DOCTOR OF MEDICINE

The pathophysiology of osteoporosis in ankylosing spondylitis

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THE PATHOPHYSIOLOGY OF OSTEOPOROSIS IN ANKYLOSING SPONDYLITIS

Submitted by

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DEDICATION

To Neelam for all her love and support, and

To Akul for bringing so much joy into our lives

STATEMENT OF ORIGINALITY

The work contained herein is my own. I personally carried out the following:

1. Screening and selection of patients and controls.
2. All clinical assessments including metrology.
3. Setting up and running the following biochemical markers of bone turnover for patients with ankylosing spondylitis:

   Serum osteocalcin,

   Total alkaline phosphatase,

   Bone alkaline phosphatase, and,

   Urinary pyridinoline and deoxypyridinoline.
4. Establishing normal ranges for the for the following markers of bone turnover:

   Osteocalcin,

   Bone alkaline phosphatase, and,

   Urinary pyridinoline and deoxypyridinoline.
5. Vertebral morphometry for all patients with ankylosing spondylitis and normal controls.

Bone sialoprotein was performed in collaboration with Tore Saxne, Department of Rheumatology, Lund University, Lund, Sweden.

Signature 

Date Feb 7, 1996

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SUMMARY

Ankylosing spondylitis (AS) is often complicated by axial osteoporosis and vertebral deformities although their relationship is not well established. The aims of this study were to determine the bone mineral density (BMD), biochemical markers of bone turnover and the prevalence of vertebral deformities in men with early AS, to establish the relationship between the markers of bone turnover, BMD and vertebral deformities, and, to assess the efficacy of etidronate on patients with AS and osteoporosis.

A detailed clinical assessment was performed to establish disease activity. Sex hormones, calciotropic hormones and biochemical markers of bone formation and resorption were measured in all patients in comparison with controls of similar age range. BMD of the lumbar spine and femoral neck was measured by dual energy x-ray absorptiometry and vertebral deformities were defined by two methods. A double blind placebo controlled study with etidronate was performed in a group of patients with AS and osteoporosis.

BMD of the lumbar spine and femoral neck was significantly reduced in the patients with AS. The prevalence of vertebral deformities in AS was 16.7% (McCloskey method) and 24.2% (Eastell-grade 1) and 7.6% (Eastell-grade 2). Compared to controls the AS patients had an increased risk for deformities. No relationship between BMD and vertebral deformities was observed. Patients with AS had significantly low osteocalcin compared with controls of similar age. No relationship was observed between markers of bone turnover and BMD or vertebral deformties. In the double blind study, patients in the etidronate group showed a significant improvement in lumbar spine BMD and disease activity compared with the placebo group.

Axial osteoporosis and vertebral deformities are features of early AS. The lack of relationship between BMD and deformity suggests that other factors in addition to low BMD may be implicated in the pathogenesis of vertebral deformities. Markers of bone turnover are not good predictors of either low bone mass or vertebral deformity. AS patients with active disease and osteoporosis may benefit from etidronate therapy.

LIST OF ABBREVIATIONS

AS	Ankylosing spondylitis
BMD	Bone mineral density
DXA	Dual energy x-ray absorptiometry
Def	Vertebral deformity
UKR	UK reference values for definition of vertebral deformity
ASR	AS reference values
CR	Control reference values
ALP	Alkaline phosphatase (total)
BALP	Bone alkaline phosphatase
BSP	Bone sialoprotein
Pyr	Pyridinoline
Dpyr	Deoxypyridinoline
25(OH)VitD	25 hydroxy vitamin D
PTH	Parathyroid hormone
FSH	Follicle stimulating hormone
LH	Luteinizing hormone
SHBG	Sex hormone binding globulin
TFI	Testosterone free index
CRP	C-reactive protein
IL-1	Interleukin 1
TNF	Tumour necrosis factor

Chapter 1.

INTRODUCTION

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1. 1. ANKYLOSING SPONDYLITIS

1. 1. 1. Historical Review

In 1982, Dastugue described a skeleton (3500 BC) in a neolithic grave in Calvados. Bony bridging of the vertebral bodies in the specimen was clearly present, but the presence of sacroiliitis was difficult to comment on, as these joints were not well preserved. The paleopathologist Sir Mark Ruffer in 1912 described a Nefermaat (2900 BC). The remains demonstrated a rigid block of bone from the mid cervical region to the sacrum. Short (1974) described 18 examples of ankylosing spondylitis (AS) derived from Egyptian sources extending from 2900 BC to 200 AD.

The general recognition of AS was promoted following the descriptions by von Bechterew in 1893 (St. Petersburg), Strumpell in 1897 (Berlin) and Marie in 1898 (Paris) [Spencer et al 1980].

1. 1. 2. Nomenclature

The term ankylosing spondylitis is derived from the Greek words *ankylos* (bent) and *spondylos* (vertebra). Various other terms have been used in the past to describe AS, including spondylitis ankylopoietica, spondylitis deformans, atrophic spondylitis, spondylitis atrophica ligamentosa, pelvospondylitis ossificans, spondylitis rhizomelica, von Bechterew's disease, Marie-Strumpell disease and rheumatoid spondylitis. The association of spondylitis with psoriasis, inflammatory bowel disorders, and enteropathic infections is now widely recognized. These conditions are said to cause secondary ankylosing spondylitis, with idiopathic AS being recognized as primary. These conditions are collectively termed seronegative spondyloarthropathies, so designated due to the absence of the IgM Rheumatoid Factor. Primary ankylosing spondylitis is the main representative of the family of spondyloarthropathies.

1. 1. 3. Epidemiology

Prevalence

Three methods have been used to determine the prevalence of AS:

1) By studying hospital in-patient records.

2) By performing population surveys.

3) By examining clinically a random population of HLA-B27 patients, mainly blood donors and extrapolating the results to the general population.

Studies of hospital records worldwide have concluded that the prevalence of AS is about 0.10% of the population. There is remarkably little variation in the data between different study groups. The prevalence rates obtained by this method have remained rather static at 0.10% - 0.20% throughout the last five decades. One explanation for this could be, that the more recent patients considered were patients with primary AS; excluding secondary seronegative spondyloarthropathy from the statistics, whereas earlier data may have possibly considered *all* patients with seronegative spondyloarthropathy.

Data collated by population surveys have reported that the highest prevalence of AS is found in the Haidas, natives of northern Canada; of whom 4.00 - 6.00% have AS (Gofton et al 1966). The prevalence of AS in Norwegian Lapps was reported to be 1.80%, with all sufferers HLA-B27 positive. Prevalence rates of AS in populations of Holland (Van der linden et al 1984), Hungary (Gomor et al 1977) and Turkey (Yenal et al 1977) were in the range of 0.10% - 0.23% of the population.

Studies to explore the prevalence of AS using blood group donors have revealed rather conflicting results. The prevalence of AS among HLA-B27 positive patients has varied from 0% to 25.00% and among HLA-B27 negative patients from 0% to 5.6%.

Valid comparisons between studies are difficult to make as the study designs varied considerably. However there does seem to exist regions with high population frequencies of AS and HLA-B27 and regions with moderate to low population frequencies.

Incidence

Carter et al (1979), on the basis of hospital based studies determined that the overall annual incidence of AS among American Whites was 6.6 cases per 100,000 inhabitants.

Sex Ratio

Population surveys collectively have shown that a sex ratio of 5 : 1 in favour of males is a likely figure. These surveys take a different view from the suggestion made by blood donor studies (Calin et al 1975) of an almost equal sex distribution of AS.

Racial distribution

The development of AS in different populations seem to be related to the prevalence of HLA-B27 in different races. For example the prevalence of HLA-B27 in the Haida Indians of North Canada is 50% (Gofton 1980), and, this population has the highest prevalence of AS, viz. 4.00 - 6.00% (Gofton et al 1966). Similarly, the prevalence of HLA-B27 in Guatemalan Indians is nil (Masi et al 1979) and clinically diagnosed AS has not been reported in this population.

Age Ratio

Although the *development* of sacral and spinal disease is usually in the late teens, the highest *prevalence* of AS may be found in the 40-45 years age group, data based on population surveys (Gran et al 1985, Thorsby et al 1971) and studies of hospital patients (Carter et al 1979). Moreover, there may be a significant decline in the prevalence rate of AS after 60 years of age (Carter et al 1979).

1. 1. 4. Etiology and Pathogenesis

HLA-B27

A strong association between ankylosing spondylitis and HLA-B27 was first discovered by Brewerton et al (1973) and Schlosstein et al (1973). A significant question is whether HLA-B27 itself confers susceptibility to disease, or whether it is merely a marker for a disease susceptibility gene closely linked with HLA-B27. Indirect evidence from clinical epidemiology (Ahearn et al 1988) and direct evidence from studies of transgenic rats (Hammer et al 1990) stongly suggests that HLA-B27 is itself the disease susceptibility gene.

HLA-B27 is a serologically defined allele of the HLA-B locus. Examination of HLA-B gene products that react with HLA-B27 typing alloantisera has led to the description of a family of allelic subtypes. The World Health Organization HLA Nomenclature Committee has named six of them as HLA-B*2701 through to HLA-B*2706. This order is based on charge determined by isoelectric focussing and not frequency (Bodmer et al 1990, Lopez de Castro 1989). The nomenclature and distribution of the HLA-B27 allelic subtypes is shown in table 1.1.4a.

Table 1. 1. 4. a. *Nomenclature and distribution of HLA-B27 subtypes.*

NOMENCLATURE	DISTRIBUTION
B*2701	Rare
B*2702	10-15%
B*2703	-
B*2704	55%
B*2705	85-90% (Caucasoids) 45% (Orientals)
B*2706	-

An association has been demonstrated between AS and the HLA-B27 subtypes. HLA-B*2701 has been seen in only a few individuals (Choo et al 1986), whereas a reasonably strong association has been found between AS and four other subtypes (Breur-Vriesendorp et al 1987). In constrast there does not appear to be an association between AS and HLA-B*2703 (Hill et al 1991). This is possibly because HLA-B*2703 has an unusual structure, as it contains a histidine at position 59 rather than tyrosine that is present in all other HLA class I molecules.

Comparison of the HLA-B27 subtypes with other class I sequences indicate that *Lys* at 70 and *Asn* at 97, are two amino acid residues that are unique to all B27 subtypes (Parham et al 1988). The HLA-B27 sequence shares a cluster of seven amino acids within the peptide binding cleft. Besides *Lys70* and *Asn97*, the others are *His9, Glu45, Cys67 Ala69 and Ala71* (Benjamin et al 1990). Besides HLA-B27, no other known class I sequence possesses more than two of these residues. It is quite possible therefore, that this portion of the B27 molecule is responsible for disease susceptibility, by allowing HLA-B27 to bind a peptide capable of trigerring the disease. The identity of such an arthritogenic peptide remains to be delineated.

The role of T cells

The only known function of the polymorphic portions of MHC class I molecules is selection of the T cell receptor (TCR) repertoire of CD8+ cells in the thymus and antigen presentation to the CD8+ cells in the periphery. Direct evidence of the involvement of CD8+ cells in the pathogenesis of the spondyloarthropathies has come from the observation made in patients of the acquired immune deficiency syndrome (AIDS), who, despite low levels of CD4+ cells develop quite aggressive reactive arthritis (Berman et al 1988, Winchester et al 1987, Calabrese 1989). The CD8+ cells react to a bacterially derived antigenic peptide bound to HLA-B27 and trigger the development of the spondyloarthropathies. It is thought that the CD4+ cells possibly function to suppress the development of reactive arthritis, because low levels of CD4+ cells in AIDS patients leads to the development of aggressive reactive arthritis.

Immunopathogenesis of the spondyloarthropathies

The role of HLA-B27 in the pathogenesis of AS still remains unclear. However, an understanding of it's function suggests ways in which it may be involved in the spondyloarthropathies.

One hypothesis suggests that infection with particular gastrointestinal or genitourinary micro-organisms can trigger an immune response with CD8+ cells. As these organisms are intracellular pathogens they would generate peptides within the infected antigen presenting host cell. The bacterial peptides that bind to HLA-B27 and are presented to the CD8+ cells are involved in the arthritogenic response. The microbial antigens besides triggering immune responses may also travel to distant tissue, including the synovium. There is evidence that Chlamydia, Salmonella and Yersinia antigens are found in synovial fluid of patients in whom these organisms are responsible for the development of reactive arthritis (Schumacher et al 1988, Merilahti-Palo et al 1991, Gransfors et al 1990).

Another hypothesis to explain the pathogenesis suggests the induction of autoreactivity to self-antigens. This is due to molecular mimicry (a small region of a human antigen is identical to amino acid sequences of proteins encoded by the triggering micro-organism) between epitopes on the infecting micro-organism and host tissues. Infection with an organism expressing an HLA-B27 mimetic peptide possibly results in priming CD8+ cells to the endogenous HLA-B27 derived amino acid sequences.

Finally, the role of HLA-B27 in the pathogenesis of spondyloarthropathies may be less direct. The HLA-B27 may only select a repertoire of TCR expressed by CD8+ cells that includes receptors uniquely capable of responding to antigens expressed by micro-organisms in a pathogenic manner.

It is important to realise that these hypotheses do not explain the immunopathogenesis of spondyloarthropathies in HLA-B27 negative individuals. None of these hypotheses are untenable and neither are they mutually exclusive of the others. It is quite likely

that a combination of the above may be involved in the development of the spondyloarthropathies.

1. 1. 5. Clinical Features

Ankylosing spondylitis (AS) is a a chronic inflammatory disorder that primarily affects the axial skeleton (spine and sacroiliac joints). Sacroiliac (SI) joint involvement is a hallmark of AS (Moll et al 1974, Arnett 1987, Resnick et al 1975). The major clinical features of AS can be divided into skeletal and extraskeletal manifestations which can be enumerated as follows:

A. Skeletal manifestations

1. Sacroiliitis
2. Spondylitis (lumbar to cervical)
3. Arthritis of girdle joints (hips and shoulders)
4. Peripheral arthritis (knees, ankles etc)
5. Spondylodiscitis
6. Enthesopathy
7. Osteoporosis

B. Extraskeletal manifestations

1. Acute anterior uveitis
2. Cardiovascular involvement
3. Pulmonary involvement
4. Cauda equina syndrome
5. Enteric mucosal lesions
6. Amyloidosis

Criteria have been laid down to facilitate the diagnosis of AS. The first set of criteria were formulated in Rome in 1961 and were subsequently revised in New York in 1966. The New York criteria were modified in 1984.

The modified New York criteria (Khan and Linden 1990) are as follows:

1. Low back pain at least 3 month's duration improved by exercise and not relieved by rest.
2. Limitation of motion of lumbar spine in sagittal and frontal planes.
3. Chest expansion decreased relative to normal values for age and sex.
4. Bilateral sacroiliitis grade 2 to 4.
5. Unilateral sacroiliitis grade 3 to 4.

Definite AS.

Unilateral grade 3 or 4 or bilateral grade 2 to 4 sacroiliitis and at least one of the 3 clinical criteria.

A basic necessity for the diagnosis of AS is the presence of sacroiliitis radiologically. The severity of sacroiliitis on the radiographs can be graded as follows:
Normal = 0; suspicious = 1; mild = 2; moderate = 3; severe (ankylosis) = 4.

Skeletal manifestations

The onset of clinical manifestations of AS is usually in late adolescence or early adulthood; onset after age 40 is very uncommon. The most characteristic clinical feature that is seen initially is low back pain, which is inflammatory in nature. This back pain has some special features that help to differentiate it from back pain that is mechanical in origin, and these are as follows:

1. Age of onset before the age of 40
2. Insidious onset
3. Duration greater than 3 months
4. Association with morning stiffness
5. Improvement with exercise

Low back pain is the initial symptom in the majority of patients. There may be mild constitutional symptoms such as anorexia, malaise, weight loss and mild fever in the early stages of the disease. Chest pain may occur due to the involvement of the

thoracic spine and the costovertebral joints, and due to enthesitis at the costosternal and manubriosternal joints. Pain and stiffness in the cervical spine is usually a late symptom, though may rarely occur early in the disease.

Sometimes the presenting symptoms may be due to involvement of the shoulder and hip joints. These joints may be involved at some stage in one third of patients (Khan et al 1984). It is now known that hip disease as a presenting feature is relatively more common when the onset of AS is in childhood or early teens. A study of the natural history of AS by Carette et al (1983), has shown that if hip involvement has not occurred in the first 10 years, it is very unlikely to occur later.

Peripheral joint involvement, other than hip and shoulder is an infrequent occurrence in AS. When present it tends to be mild and usually resolves without any residual joint damage. Cohen et al (1982) have shown that peripheral joint disease can occur occasionally after the axial disease has become inactive.

Enthesitis is inflammation of the entheses (point of insertion of tendons and ligaments into bone). The development of enthesitis can cause tenderness on pressure at various sites like the costosternal junctions, spinous processes, iliac crests, greater trochanters, ischial tuberosities, tibial tubercles or heels.

Spinal osteoporosis and vertebral fractures has been reported in patients of severe AS of long duration, as a result of ankylosis and immobility (Ralston et al 1990). A reduction in bone mineral density in the spine and femoral neck has also been reported in young patients of AS who had minimal clinical and radiological disease (Will et al 1989). Further details of osteoporosis and vertebral fractures in AS are described in later sections (1.6 and 1.7 respectively).

Extraskeletal manifestations

Acute anterior uveitis (iritis or iridocyclitis) is the commonest extraskeletal manifestation in AS. It occurs in 25-30% of patients (Rosenbaum 1992) and is relatively more common in HLA-B27 positive than HLA-B27 negative patients with AS. The uveitis is usually unilateral, and is characterized by pain, increased lacrimation, photophobia and blurred vision. The inflammation is non-granulomatous (Rosenbaum 1992). The uveitis has a strong tendency to recur, quite frequently in the contralateral eye and may occasionally be the presenting sympton.

Cardiovascular involvement due to AS is a rare complication. Ascending aortitis, aortic valve incompetence, conduction abnormalities, cardiomegaly and pericarditis have been observed (Bergfeldt et al 1988; Graham et al 1958). Pleuropulmonary involvement has been observed as a rare and late manifestation of AS. Rosenow et al (1977) have reported an incidence of 1.3%. The commonest pulmonary abnormality observed is a slowly progressive pulmonary fibrosis of the upper lobes of the lungs. It is usually bilateral, cavitation may occur with colonization of these cavities by micro-organisms of the Aspergillus species.

A slowly progressive cauda equina syndrome has been reported in patients with advanced AS (Tullous et al 1990). The development of amyloidosis is a very rare complication of AS (Cruikshank 1960, Jayson et al 1971).

Other rare complications of AS include IgA nephropathy (Bruneau et al 1986) and the development of asymptomatic mucosal lesions in terminal ileum and colon. These have been detected on endoscopic studies in 30-60% of patients with AS and reactive arthritis, suggesting that inflammation of the gastrointestinal tract may have a role in the pathogenesis of 'primary' AS not associated with clinical inflammatory bowel disease (De Vos et al 1989).

1. 1. 6. Pathology

Pathology of the Enthesis

Much of the skeletal pathology in AS can be explained on the basis of the inflammatory changes at the entheses (ligamentous and tendinous attachment to bone). The initial inflammation is an erosive process followed by healing and new bone formation (Ball 1971). Ball has demonstrated that the initial erosive phase is patchy and new bone is laid down in fibrous tissue without preceding cartilage formation. The final outcome therefore, is an irregular bony prominence with sclerosis of the adjacent cancellous bone.

The inflammatory and destructive enthesopathy that occurs initially, is followed by a healing process. This leads to the formation of new bone and subsequent ankylosis between adjacent vertebrae and is responsible for the characteristic spinal changes in AS (Ball 1971). The formation of syndesmophytes is usually as an end result of enthesopathy at the anterior or antero-lateral attachment of the outer annulus at or just below the junction of the annular flange and the corner of the vertebral body, and sometimes at both sites. Enthesopathic repair results in the formation of lamellar bone, and it is the extension of this lamellar bone across the outer layers of the annulus leads to the visual appearance of syndesmophytes. The stimulus to the progressive growth of syndesmophytes however, remains speculative.

It is noteworthy that extensive lesions of the disc-bone junction of the vertebral end-plate are probably unique to AS and are said to occur most frequently in spines showing extensive syndesmophytosis (Cawley et al 1972). It is speculative whether the primary etiology of the vertebral end-plate pathology is inflammatory or mechanical.

Pathology of synovial joints

Changes in synovial joints in AS are similar to the changes seen in rheumatoid arthritis (Cruikshank 1951, Cruikshank 1971, Cooper et al 1981) The changes include villous proliferation of the synovial tissue and hyperplasia of the synoviocytes with occasional

multinucleate cells. Infiltration by lymphocytes and plasma cells is seen, but the formation of nodular aggregates is less than that seen in rheumatoid arthritis.

A change unique to AS is extensive bony bridging across the joint accompanied by preservation of extensive areas of articular cartilage. This results in the retention of the joint space in a joint that is completely fused. Extensive ossification of the joint capsule, ligaments and meniscal structures resulting from enthesopathy is quite commonly seen.

Involvement of the apophyseal and sacroiliac joints is of special importance in AS.
The apophyseal joints may be involved in two ways:

a) There may be inflammatory and erosive synovitis, and
b) There may be an enthesopathy at the capsulo-ligamentous attachments and capsular ossification leading to ankylosis. This will lead to the apophyseal joint getting encased in an outer bony shell with an otherwise normal joint; these capsular changes being the apophyseal equivalent of syndesmophytes (Ball 1971).

Pathological changes of fibrosis and ankylosis may be seen in 'normal' sacroiliac joints in the absence of AS (Horwitz et al 1940; Resnick et al 1975). Hence the only change in sacroiliac joints specific to AS is capsular ossification producing an outer shell of bone as is seen in the apophyseal joints. Ossification of interosseous ligaments also contribute to the fusion between the ilia and sacrum in AS. The 'erosions' of the sacroiliac joints seen radiographically in AS, has been attributed to the irregular progression of endochondral ossification (Ball 1971).

Inflammatory changes have also been noted in cartilaginous joints like the manubriosternal joint and the symphysis pubis in patients with AS. However there is no evidence presently to suggest that these changes are unique to AS as they have also been noted in patients with rheumatoid arthritis (Cruikshank 1951).

1. 1. 7. Management of Ankylosing Spondylitis

The goals of management are to restore and maintain posture and movement as near normal as possible. The mainstay of therapy in AS is physiotherapy. However, as with all rheumatic disorders early diagnosis and patient education play a very important role in the management of AS. As soon as a conclusive diagnosis is made based on clinical , laboratory and radiological parameters, the patient must be given a clear description of the nature of spondylitis.

Initially the aim should be to reduce the patient's pain and stiffness. This is achieved with the help of non steroidal anti-inflammatory drugs (NSAIDS). The choice of NSAIDS is based on it's efficacy and tolerance in each individual. In a few cases a second line agent may also be required. A meta analysis by Ferraz et al in 1990 suggests that Sulphasalazine is possibly the most effective drug in AS. There is also some indication that clinical improvement of AS is accompanied by the lowering of acute phase reactants like CRP, plasma viscosity and ESR. Long term corticosteroids have little part to play in the management of AS. However, bolus doses of methylprednisolone in pulses have been used with success in patients with very severe symptoms.

The cornerstone of therapy is physiotherapy. The role of physiotherapy in treatment is essentially to alleviate pain by using a variety of pain relief methods like short wave diathermy, interferential therapy, local heat or cold and local ultrasound, and by teaching the patient a program of exercises aimed at maintaining posture. It is always a good idea to incorporate as much exercise as possible into the patients' lifestyle, encouraging recreational exercises such as swimming and badminton. An objective record of the patients posture is useful. Serial recordings of the status of the cervical spine (Tragus-wall measurement), thoracic spine and chest wall (chest expansion), lumbar spine [Macrae's modification (1969) of the Schober's test], hip movements (intermalleolar distance) and enthesopathy index associated with visual analogue scales for pain and stiffness are useful.

1. 2. CELLULAR BASIS OF BONE TURNOVER

1. 2. 1. Bone Cells

Most of the pathologic change of bone is related to the processes of bone formation and resorption. These processes are carried out by two groups of specialized cells, the osteoblasts and the osteoclasts.

Osteoprogenitor cells

Progenitor cells are constituents of regenerating tissue. These cells may undergo asymmetric division, resulting in another progenitor cell and a differentiated or predifferentiated cell. In bone these cells are called osteoprogenitor cells.

Friedenstein (1973) divided the osteoprogenitor cells into two types:
i) Determined osteoprogenitor cells (DOPC), and,
ii) Inducible osteoprogenitor cells (IOPC).

The DOPC can form bone in Millipore filter chambers implanted into animals. The Millipore chambers allow free diffusion of molecules but no penetration of cells (Friedenstein 1976, Ashton et al 1980, Bab et al 1986). The IOPC on the other hand can form bone in Millipore chambers only in the presence of 'inducing' influences (Friedenstein 1968, Owen 1980). DOPC are found in the periosteum and bone marrow and are thought to be reticular cells next to endosteal surfaces (Menton 1982). The IOPC have been shown in thymus, spleen and lymph nodes (Friedenstein 1976). It is not clear in what way the IOPC differ from the DOPC and what the mechanisms of induction are. It is possible the inducing substance is a peptide. The progenitor cells are usually quiescent and tend to get activated during bone modelling and remodelling and in response to injury.

Preosteoblasts

Preosteoblasts are in a transitional state between the osteoprogenitor cell and the differentiated osteoblast. This transition state lasts for 2-3 days. The preosteoblasts have the capacity to proliferate and differentiate into osteoblasts. The regulatory forces

for this proliferation are however unknown. The preosteoblast is incapable of producing bone matrix. It is possible that locally produced growth and differentiation factors like insulin like growth factor-1 and transforming growth factor beta promote osteoblast maturation (Heine et al 1987). The preosteoblasts lie close to the osteoblast on the side away from the bone surface.

Osteoblasts and osteocytes

The primary difference between the osteoblast and the osteocyte is their relationship to the environment, and, the secondary difference is in their structure and function. The osteoblast is located closely apposed to the bone surface. The osteocyte is surrounded by mineralized matrix. Nijweide et al (1981) suggested an intermediate stage between these two, that is cells surrounded by unmineralized bone matrix and called this the osteocytic osteoblast. Palumbo (1986) named this the osteoid-osteocyte; the cell that is completely surrounded by mineralized matrix she called pre-osteocyte. All these cells (osteoblasts, osteocytes, osteoid-osteocytes, osteocytic osteoblasts and pre-osteocytes) are capable of forming bone matrix. These cells are in different stages of development. When the osteoblast is not synthesizing matrix they change shape and are called inactive osteoblasts or resting osteoblasts and are found commonly on the surface of adult bone.

The osteoblasts are derived from two sources: undifferentiated primitive mesenchymal cells and differentiated chondrocytes. Morphologically the osteoblast is cuboidal in shape and have three prominent structures that reflect the main function of the cell, which is, active synthesis of the proteins and polysaccharides of the bone matrix. These three structures are an eccentric nucleus, the rough endoplasmic reticulum and the golgi apparatus (Holtrop 1975). The osteoblasts are connected to each other by means of gap junction (Doty 1981, Miller et al 1980). The osteoblasts and osteocytes are also connected by numerous processes running through canaliculi in the bone matrix. Thus all the bone cells are connected to each other to form a functional unit.

A characteristic feature of osteoblastic activity is the secretion of various products and enzymes. These include collagen (type I), collagenase, non-collagenous proteins like osteocalcin, proteoglycans, plasma proteins (albumin, globulin, lactoferrin), phosphoproteins (osteonectin, sialoprotein or osteopontin), bone morphogenetic protein, matrix gla protein, thrombospondin (an attachment protein) alkaline phosphatase and prostaglandins. Another feature of osteoblasts is their ability to respond to tissue specific hormones that influence bone metabolism and calcium homeostasis. These features of the cells of osteoblastic lineage play a significant role in the regulation of bone formation and resorption.

The osteocytes are elongated cells and are oriented with their long axis parallel to the collagen fibres around them. Young osteocytes that have just been encased in mineralized matrix resemble osteoblasts in every way morphologically. However, there are significant differences in morphology once the osteocyte matures, more mineral is laid down and the cell becomes located deeper in bone. The cell becomes smaller, the cytoplasm decreases in amount and the nucleus becomes the most prominent feature. The osteocyte is said to go through three stages: the formative stage, the resorptive stage and the degenerative stage. Active resorption by the osteocytes seems to require certain metabolites and is termed as 'osteocytic osteolysis' (Belanger 1967, Belanger et al 1969). Jande (1972) and Krempien et al (1976) confirmed the resorptive action of osetocytes in animal experiments. One can therefore conclude that osteocytes besides their well documented bone formative effects also have a resorptive function.

Osteoclasts and other cells

Osteoclasts are considerably larger than the osteoblasts and are found on the surface of bone. These cells can take many shapes, which suggests cell movement.The osteoclast contain many nuclei and extremely prominent nucleoli. These cells do not have as much rough endoplasmic reticulum as the osteoblasts and therefore there is less production of protein for secretion. The most characteristic feature of an osteoclast is the presence of "ruffled borders" when the cell is resorbing bone (Scott and Pease 1956). It is noteworthy that the structure of the cell membrane in the ruffled border is somewhat different from the rest of the plasma membrane, being coated with fine

bristle like structures that are evenly spaced and projecting perpendicularly into the cytoplasm (Kallio et al 1971). Always accompanying the ruffled border is a specific area along the cell membrane at the periphery of the ruffled border. This area is termed the "clear zone". The osteoclast is helped in its attachment to the bone surface by the clear zone. Once an osteoclast has attached itself to the bone, the ruffled border becomes highly motile. Ruffled borders and clear zones are never seen away from the bone surface. It is not known what happens to these areas once the osteoclast stops resorbing and moves away from the bone surface.

It has been shown that the osteoclast can also make contact with neighbouring cells. Soskolne (1979), observed that the osteoclast can make contact with endothelial cells with the help of small processes that reach out from the osteoclast towards the endothelial cell. Osteoclasts can also make contact with pericytes.

There is a general agreement that osteoclasts are derived from the haematopoietic stem cell and should be included in the haematopoietic cell family.The relationship of the osteoclast with the mononuclear phagocyte pathway are still unclear. One body of evidence suggests that the osteoclasts are full blown members of the mononuclear phagocyte system, whereas, the other body of evidence cannot find any connection at all between the osteoclast and the mononuclear phagocytes.

The osteoclasts are stimulated by parathyroid hormone (PTH). PTH can also increase the number of functioning osteoclasts (Holtrop et al 1974, King et al 1978, Holtrop and Raisz 1979). The number of nuclei per cell also increases after exposure to PTH (Wezeman et al 1979, Addison 1980). Stimulation of bone resorption in cultures by PTH can be reversed by calcitonin and this is accompanied by a decrease in the number of active osteoclasts (Holtrop et al 1974). The number of osteoclasts in vivo can also increase after exposure to $1,25(OH)_2D_3$ (Holtrop et al 1981, Marie and Travers 1983) and prostaglandins (VanderWiel 1979).

Glowacki et al (1981) noted from experiments with rats that multinucleated giant cells without the ruffled borders or clear zones of the osteoclasts were capable of resorbing

bone. She in a later study demonstrated that the multinucleated giant cells could develop ruffled borders and clear zones and showed other characteristics of osteoclasts, such as the presence of tartrate resistant acid phosphatase (Glowacki and Cox 1986). It is now known that other cells like monocytes and macrophages also have the ability to resorb bone (Kahn et al 1978, MacArthur et al 1980).

The mononuclear cells appear to have three functions in bone resorption:
a) Resorb bone by ingesting partially degraded collagen fibrils (Heersche 1978).
b) Release factors locally that activate osteoclasts (Yoneda and Mundy 1979).
c) Serve as precursors for osteoclasts.

One can therefore say that osteoclasts are cells of bone resorption, but they are not alone.

1. 2. 2. Bone Matrix

Collagen

Type I collagen is the most abundant protein of the body and is found in a number of diverse extracellular matrices. Only two collagen types, type I and type V have been shown to be synthesized by osteoblasts. Bone therefore contains almost exclusively type I collagen with some type V (Broek et al 1985, Bronckers et al 1986).

The main characteristics of collagen are its triple helical domains. Glycyl residues occupy the centre of the helix, while the other positions (X and Y) of the helix are often occupied by prolyl and hydroxyprolyl residues. Nimni (1983) has comprehensively described the complex sequence of events leading from the transcription and translation of events of the collagen genes to the organization of collagen fibers in the extracellular matrix and the subsequent deposition of mineral.

These events can be briefly described as under:

* transcription of genes for type I collagen located on chrosomes 7 and 17, coding for specific $\alpha 1$ (I) and $\alpha 2$ (I) chains of nascent bone type I pro-

collagen,

* translation of mRNA on osteoblast ribosomes,
* post-translational glycosylation and hydroxylation of proline and lysine,
* assembly of two α1 (I) chains and one α2 (I) chain into a triple helix,
* translocation of the procollagen from site of synthesis to the cell membrane, secretion of the procollagen,
* cleavage of the NH2-terminal and COOH-terminal extension peptides,
* cross-linking and specific interaction with other extracellular macromolecules, such as fibronectin, osteonectin and proteoglycans,
* mineralization,
* degradation and removal during bone resorption.

The preservation of the three dimensional relationships within collagen are required for calcification, and may well be important in its catabolism by collagenase (Harris and Farrell 1972, Vater et al 1979). It is noteworthy that the triple helix is resistant to all proteolytic enzymes but collagenase.

A recent thought is that collagen can only perform its function as an 'alloy' of different collagen types and other molecules, such as proteoglycan II (PG-II) (Scott and Haigh 1985). As an alloy it yields the proper fibrils to interact with various components of the matrix, thereby being able to participate in the chain of interactions that is required to maintain the architecture of the extracellular matrix. Unravelling the nature of these interactions should contribute to our understanding of bone biosynthesis and pathology.

Collagenase

Collagenase is an enzyme that is primarily associated with the degradation of collagen under physiological conditions. It was first identified in tadpole tissue (Gross et al 1962, Nagai et al 1966) and Fullmer and Lazarus in 1967 found bone collagenase to be a specific neutral metalloproteinase. It appears to resemble the enzymes found in various other organs and tissues which degrade interstitial collagen. In most tissues collagenase is synthesized *de novo* and is not stored. A distinct property of bone

collagenase is its interaction with heparin. Studies have now confirmed an increased production of collagenase by explanted bones in response to heparin (Shimizu et al 1969, Vaes 1972). The effect of heparin on bone collagenase occurs at the level of target organs or effector cells of heparin.

The cell specificity of bone collagenase has been a controversial issue. Sakamoto and Sakamoto (1984) studied collagenase synthesis in the mouse clonal osteoblastic (MC3T3-E1) cells and demonstrated conclusively the capacity of osteoblastic cells to produce collagenase *in vitro*. These findings were confirmed by Heath et al (1984) and Otsuka et al (1984, 1984a). On the basis of these data it may be concluded that the primary cells of collagenase production in bone tissue are the osteoblastic cells.

The activity of collagenase in vivo is regulated by collagenase inhibitors, parathyroid hormone, IL-1 (Mizel et al 1981) and physicochemical and pharmacologic agents. α2-macroglobulin is the principle circulating inhibitor of serine-, thiol-, carboxyl-, and met allo-proteinases (Barrett et al 1973).

Osteocalcin

Osteocalcin or Bone Gla-protein (BGP) is a small vitamin K dependent, calcium binding peptide synthesized almost exclusively by osteoblasts and odontoblasts. It has been isolated from more than 12 different species. The mature protein contains 46-50 amino acids in a single peptide chain whose molecular weight ranges from 5200-5900. Osteocalcin binds tightly to calcium in hydroxyapatite crystals and weakly to free calcium ions. The gene coding for human osteocalcin has been localized to the long arm of chromosome1 by somatic cell hybridization techniques (Puchacz et al 1989).

The precise function of osteocalcin is not known. The molecule demonstrates biological properties that suggest a role in the formation of bone.

The biological functions of osteocalcin can be summarized as follows:

i) It can inhibit the precipitation of hydroxyapatite from supersaturated systems.

ii) It can inhibit the conversion of brushite to hydroxyapatite.

iii) It is an effector molecule for $1,25(OH)_2D_3$ action.

iv) It is an inhibitor for leucocyte elastase.

v) It is a chemoattractant for peripheral blood monocytes (Malone et al 1982, Mundy and Poser 1983).

Cellular regulation of osteocalcin to a large extent is by $1,25(OH)_2D_3$ and growth factors like basic fibroblast growth factor. The doses of $1,25(OH)_2D_3$ that stimulate osteocalcin synthesis also depress collagen synthesis and repress expression of alkaline phosphatase activity and cell proliferation. Osteocalcin synthesis is restricted to osseous tissues, although many cell types support vitamin K dependent carboxylation (Hauschka et al 1989). The human bone content of osteocalcin is 10% of the amounts found in other species (Lian and Gundberg 1988). Compared to alkaline phosphatase activity, osteocalcin synthesis starts later and persists longer.

A portion of newly synthesized osteocalcin enters the systemic circulation while the rest finds its way to osseous tissues. This circulating fraction can be readily detected and quantified by RIA and ELISA. In the adult human, an estimated one third of *de novo* synthesis is destined for the circulation. The half life of osteocalcin in the circulation is five minutes. Early proteolytic digestion may occur in the liver (Melick et al 1988) whereas osteocalcin is cleared largely by the kidney (Price et al 1981). The precise mechanism for the filtering of the peptide and its degradation in the kidney is not well known though it is clear that the constituent Gla residues are excreted unchanged in the urine.

Serum osteocalcin shows a circadian rhythm, with a peak at about 1400 hours and a nadir at about 1200 hours. There are several reasons to believe that changes in serum osteocalcin concentrations reflect changes in bone formation (Lian and Gundberg 1988). These reasons are:

* It is the osteoblasts that produce osteocalcin and not osteoclasts.
* Osteocalcin in the serum is derived from newly synthesized osteocalcin, and not from previously degraded matrix.
* A close correlation exists between serum osteocalcin and histomorphometry of

bone mineral.

In adults, serum osteocalcin levels fall to values between 2 and 12 ng/ml after puberty. Differences have been noted in the values of osteocalcin between males and females, particularly in females in the period immediately after menopause. In general however, variations in osteocalcin values with age and sex parallels the changes in bone turnover, with increased values during puberty and menopause, when there is increased bone turnover. In postmenopausal osteoporosis, characterized by increased bone turnover, osteocalcin levels have been found to be high (Civitelli et al 1988), with no significant correlation observed between serum osteocalcin and bone mineral density in either the femoral neck or lumbar spine. Dannucci et al (1987) demonstrated high osteocalcin levels in oophorectomized dogs in whom the trabecular bone remodelling was similar to that observed in high turnover postmenopausal osteoporosis.

In osteoporosis, when there is both trabecular and cortical bone loss, reflecting both an increase in PTH mediated bone resorption and a defect in bone formation, the osteocalcin levels have been found to be low (Cooper et al 1989), supporting the hypothesis that there is decreased osteoblast function in this type of osteoporosis. Low levels of osteocalcin have also been demonstrated in rheumatoid arthritis (Ekenstam et al 1986) and ankylosing spondylitis (Franck et al 1993, Ekenstam et al 1986).

One can therefore say that serum osteocalcin appears to reflect bone formation rates in patients with osteoporosis, and is likely be of value in assessing the response to treatment.

Sialoprotein

Sialoprotein is a phosphorylated glycoprotein and has an acidic nature. The acidic nature of this protein confers on it a high capacity to bind calcium, a situation not altered by acid hydrolysis. Two sialoproteins have been identified, sialoprotein-I (also called osteopontin) and sialoprotein-II also referred to as bone sialopeotein (BSP), (Fisher et al 1987, Franzen et al 1985, 1986). Osteopontin has been identified in the golgi apparatus of osteoprogenitor cells, osteoblasts and osteocytes from

unmineralized endochondral and membranous bones of newborn rats (Yoon et al 1987). This bone specific phosphoprotein has not yet been detected in odontoblasts or in non-osteogenic mesenchyme. Interestingly, the amino acid sequence of this protein (Arg-Gly-Asp) matches the sequence identified in cell attachment proteins like fibronectin (Oldberg et al 1986). Its function may be like fibronectin's.

BSP is more heavily sialylated than osteopontin and contains 50% carbohydrates (Fisher et al 1983). BSP constitutes some 12% of the non-collagenous proteins in bone, which makes it a major component of this group of proteins. It may be designated a phosphoprotein since some 30% of the serine residues are phosphorylated. Although the molecule has not yet been cloned at the molecular level, its amino-terminal sequence has been determined (Fisher et al 1987). The function of this protein is yet to be determined, although a role in mineralization has been proposed. There is growing appreciation that sialoproteins along with other phosphoproteins which favour calcification may endow bone with chemoattractants for the precursors of osteoclasts.

Matrix-Gla Protein

Matrix-Gla protein (MGP) is unrelated to osteocalcin. It appears in developing bone at an earlier developmental stage (Price et al 1983). MGP appears to be strongly associated with the bone morphogenetic protein (BMP). Price proposed that the MGP might either serve as a carrier for BMP, or be involved in anomalous patterns of calcification observed when warfarin treated rats are treated with calcitriol.

Bone Morphogenetic Protein

Bone morphogenetic protein (BMP) is a low molecular weight protein.The identity of cells that produce BMP has not yet been firmly established. The protein is diffusible and acts to stimulate the proliferation of mesenchymal cells. The integrity of BMP is maintained in collagenase digests, but it can be degraded by proteolysis and reducing agents (Harakas 1984). It has an osteo-inductive function.

Osteonectin

Osteonectin is the most abundant of the phosphoproteins and glycoproteins present in the matrix of bone. It is an acidic phosphoprotein because it is rich in aspartic and glutamic acid residues, and it is a glycoprotein with glucosamine, galactosamine and sialic acid residues. Both native and denatured osteonectin bind tightly to hydroxyapatite (Romberg et al 1986) and native osteonectin has the ability to bind calcium ions (Romberg et al 1985). Osteonectin can also bind to type I collagen and albumin. Osteonectin can also inhibit hydroxyapatite seeded crystal growth (Romberg et al 1986). Besides being found in bone, studies have also demonstrated the presence of osteonectin in platelets (Stenner et al 1986, Kelm and Mann 1990). Although the function of this protein in bone and platelets still needs to be fully elucidated, it appears to be a marker of mature matrix producing osteoblasts by virtue of its localization to bone trabeculae and newly formed osteoid.

Proteoglycans

Proteoglycans constitute approximately 5% of the noncollagenous matrix of bone. Proteoglycans have a central protein core to which are attached glycosaminoglycans and oligosaccharides. Rosenberg et al (1985) named the two most abundant proteoglycans in bone as PG-I and PG-II. The function of proteoglycans and their role in mineralization are not fully clear.

1. 2. 3. Regulation of the Resorption and Formation of Bone

Mechanisms of resorption

Until a few years ago most investigators could describe with confidence the separate roles of the osteoblasts in bone formation and of osteoclasts in bone resorption. Now however, bone formation is thought to be intimately coupled with bone resorption, and there is increasing evidence to suggest that bone resorption is a dynamic event which not only involves the osteoclast but also the cells of the osteoblastic lineage.

There is no doubt that the osteoclast is the major cell responsible for bone resorption. However, other cells of bone, particularly cells of the osteoblastic lineage and

macrophages also possess and secrete proteolytic enzymes. This, coupled with the lack of demonstrable collagenase in osteoclasts, has led to the suggestion that these other cells may act as alternative or accessory bone resorptive cells. However, it has not been possible to demonstrate whether these cells are capable of the excavation of bone, a function which seems to be unique to the osteoclast. Besides, the osteoclast effects this without any assistance from the other cells. Another fact that favours the involvement of other cells besides osteoclasts in bone resorption, is that osteoclasts cannot excavate unmineralized bone, only being active if the unmineralized surface was first removed by collagenase secreted by the osteoblasts (Chambers and Fuller 1985).

The osteoclasts resorb bone by forming a sealing zone of close adhesion between the osteoclast and bone (Miller et al 1984). Within this sealing zone, the osteoclastic plasma membrane develops 'ruffled borders' the size of which is proportional to the resorbing activity. There is now considerable evidence that osteoclasts resorb bone through secretion of enzymes and proteins in the sealed space between bone and the ruffled border. In this extracellular microenvironment, bone is degraded by acid hydrolase activity. The resorptive hemivacuole is the functional equivalent of an extracellular phagolysozome, a speciality of the osteoclast. Baron et al (1985) demonstrated that the osteoclast actively acidifies beneath the ruffled border. However, the natural substrates, biochemical characteristics and role in bone resorption of the majority of the osteoclastic acid hydrolases is unknown.

Osteoclastic resorption tends to occur with a certain degree of site selectivity. As the osteoclast is essentially a wandering cell, it can only reach bone resorbing areas by receiving well directed 'traffic signals'. Cells of the osteoblastic lineage appear well equipped to provide the morphogenetic information required for osteoclastic localisation. Thus osteoblastic cells may be able to recruit, localize, activate, regulate and terminate osteoclastic bone resorption. A similar explanation can be given for substances like PTH which are systemic stimulators of osteoclastic bone resorption. PTH increases both the number of osteoclasts and the activity of pre-existing osteoclasts. Rodan and Martin (1981) have demonstrated the presence of PTH receptors on osteoblastic cells and this was also reported by most (O'Grady and

Cameron 1971, Silve et al 1982, Rouleau et al 1986), but not all (Rao et al 1983) investigators.

Another method by which the osteoblast functions to assist the osteoclast in resorbing bone is by removing unmineralized organic material from the surface in response to PTH. This exposes underlying mineral, and bone modified in this manner develops an increased susceptibility to osteoclastic resorption which can only be abolished by demineralisation (Chambers and Fuller 1985). The osteoblasts can achieve this removal of unmineralized matrix to a large extent with the help of collagenase that they secrete. Collagenase can digest the organic unmineralized layer, enabling resorption of bone. The osteoclastic activity can also be optimized by growth factors produced by the osteoblasts like transforming growth factor-beta (TGF-b), interleukin-1 (IL-1), tumour necrosis factor (TNF), epidermal growth factor (EGF) and prostaglandins.

Thus, although one may say that the phenomena of bone formation and resorption are coupled, the coupling phenomenon has essentially been rewritten in the last decade.

Mechanisms of bone formation

In spite of extensive research over many years, surprisingly little is known about the mechanisms involved in the formation of bone. Few reliable means to stimulate bone formation *in vitro* (or *in vivo*) exist. Therefore, there have been difficulties in manipulating the process of bone formation in vitro.

The formation of bone involves three complicated steps:

1) Proliferation of progenitor cells, and their differentiation into active, mature, osteoblasts.
2) The production of bone matrix, and,
3) Calcification of the bone matrix.

The first two have already been dealt with in the preceding sections. The calcification of bone matrix involves cell regulated concentration and deposition of, primarily calcium, and phosphate at the site of calcification. Understanding of the mechanism of

in vitro mineralization was made by Tenenbaum and Heersche (1982), when they added beta-glycerophosphate to the culture medium, the rationale being the availability of organic phosphate around cells with a high alkaline phosphatase activity. Calcification has been demonstrated to occur in the presence of organic phosphates in experimental animals. The precise mode of action of organic phosphates, especially beta-glycerophosphate is yet to be established.

Factors regulating bone resorption and formation

i) Parathyroid hormone (PTH)

The effect of PTH on bone can be catabolic as well as anabolic. The rapid catabolic effect is mainly via an increase in bone resorption by osteoclasts (Raisz 1963), and to a lesser extent by inhibition of bone formation by osteoblasts (Dietrich et al 1976, Kream et al 1980). The anabolic effect is relatively slow and involves stimulation of bone formation.

Following the effect of PTH on bone, there is a net release of calcium, leading to hypercalcaemia. This is associated with a breakdown of extracellular organic matrix. This breakdown of matrix is paralleled by an increase in proteolytic and lysosomal enzymes, viz. acid phosphatase and beta-glucuronidase, as well as carbonic anhydrase (Vaes 1968). Carbonic anhydrase appears to be localized along the ruffled border of the osteoclast and may contribute to the dissolution of calcium phosphate crystals by acidifying the milieu. Inhibitors of carbonic anhydrase have been shown to block basal and PTH mediated bone resorption (Minkin and Jennings 1972).

Besides mediating osteoclast induced bone resorption, PTH can also inhibit the synthesis of matrix proteins by osteoblasts. Among these proteins are collagen and osteocalcin. The inhibitory effects of PTH on collagen synthesis are said to occur in deeper layers of bone containing osteoblasts and not in the periosteal layer.

As discussed earlier, PTH also induces morphological changes in the osteoblasts and osteoclasts, by virtue of receptors on these cells.

The anabolic effects of PTH were elucidated by Selye in 1932. In experimental models this anabolic effect has been easiest to demonstrate when PTH is used in low doses and intermittently. This effect is associated with increased bone formation, with an increase in collagen synthesis, and alkaline phosphatase activity. The possible mechanisms postulated are a proliferation of the precursor cells by PTH, an increase in the number of sites for the remodeling of bone and an increase in the circulating levels of PTH which would lead to an increase in the osteoblast precursor cells and ultimately to an increase in bone formation.

The effect of PTH on the regulation of bone formation and resorption can also be mediated by other factors and hormones like, cAMP, steroid hormones and $1,25(OH)_2D_3$.

ii) Vitamin D

Although the primary physiological role of vitamin D appears to be the promotion of bone growth and mineralization, there is no firm evidence to support this. However by increasing the extracellular fluid concentrations of calcium and phosphate, vitamin D can indirectly promote bone mineralization. However $1,25(OH)_2D_3$ is a potent direct stimulant of bone resorption (Maierhofer et al 1983) and can enhance the maturation of osteoclast precursors (Takahashi et al 1986). $1,25(OH)_2D_3$ can also stimulate osteocalcin production from the osteoblast (Markowitz et al 1987) as well as the production of IL-1 (Amento et al 1984). However no effect of vitamin D on prostaglandin production has been demonstrated. No osteoclast receptor for vitamin D has been identified.

The initial effect of $1,25(OH)_2D_3$ on collagen synthesis is also inhibitory (Raisz et al 1980, Bringhurst et al 1982). The anabolic effects of vitamin D on bone formation are not as clearly established as that of PTH. Administration of physiologic doses of vitamin D or its metabolites may lead to an increase in bone mass under certain conditions, but this has not been a consistent finding. Administration of toxic doses of

$1,25(OH)_2D_3$ to experimental animals however results in a stimulation of bone matrix production with impaired mineralization (Boyce et al 1983).

The effects of vitamin D and its metabolites seems to be principally on the resorption of bone mediated by osteoclasts.

iii) Calcitonin

Calcitonin is a hormone secreted by the parafollicular C cells of the thyroid gland, in response to an increase in ionized calcium levels. The osteoclast is the major target for the action of calcitonin. This hormone can inhibit osteoclastic resorption at very low concentrations. There is evidence to suggest that the hormone has a direct effect on the osteoclast itself. Holtrop et al (1974) have shown that pharmacologic doses of calcitonin can completely inhibit osteoclastic bone resorption.

The morphological and functional changes that take place in the osteoclast in response to calcitonin are as follows:

1) A rapid decrease in numbers of circulating osteoclasts (Baron et al 1981, Hedlund et al 1983).
2) Loss of ruffled border of osteoclasts (Kallio et al 1972, Singer et al 1976).
3) Physical separation of the osteoclast from the underlying bone (Kallio et al 1972).
4) Inhibition of osteoclastic Na+ K+ ATPase activity (Akisaka et al 1986)
5) Relocation of the enzyme carbonic anhydrase II (Anderson et al 1982).

Chambers and Magnus (1982), and Chambers and Moore (1983), demonstrated a dramatic change in the morphology and behaviour of rat osteoclasts in response to salmon calcitonin in picogram/ml concentrations. The rapidity with which the osteoclasts act in cultures when separated from other cells suggests that calcitonin acts directly on osteoclasts. Cyclic AMP (cAMP) generation seems to be involved in the inhibition of osteoclasts by calcitonin. Calcitonin increases the cAMP content of bone (Murad et al 1970). cAMP tends to cause an immobility of the osteoclasts,

although osteoclasts per se are more prone to resorption inhibition than motility inhibition.

The physiological role of calcitonin is unknown. The action on osteoclasts is possibly the only action of physiological significance. Besides, the osteoclasts are the only cell types in bone shown to possess calcitonin receptors or responsiveness. The hormone also acts on the kidney to inhibit normal calcium excretion, and to increase $1,25(OH)_2D_3$ production.

iv) Prostaglandins

Prostaglandins are derivatives of arachidonic acid metabolism. The action of these agents depend on their concentration. They can regulate bone formation and resorption.

The effect of prostaglandins on bone resorption has been studied *in vivo* (Goodson et al 1974) and *in vitro* (Hirata et al 1983) experiments. The same prostaglandins that stimulate resorption in organ cultures tend to inhibit resorption in small doses. This is possibly as a direct effect of prostaglandins on the osteoclasts themselves. Like calcitonin, prostaglandins inhibit enzyme release by the osteoclasts and cause cAMP production by the osteoclasts. The direct effect of prostaglandins on osteoclasts is possibly an inhibitory one.

The prostaglandins are also produced by the osteoblasts (Feyen et al 1984, Rodan et al 1986). It may be that prostaglandins are the means by which osteoblasts mediate some of their osteoclast inhibitory effects. Since prostaglandins also have a direct effect on the osteoblasts, they may promote bone formation. It has been shown that the prostaglandins, by their action on the osteoblast, can lead to cortical bone hypertrophy (Stern et al 1985) and hyperostosis (Ueda et al 1980), fracture healing and bone remodeling (Dekel et al 1981).

v) Miscellaneous

There are other factors and hormones that play a role in the regulation of bone resorption and formation. These include bone-cell derived growth factors which may be transforming, like transforming growth factor-beta (TGFβ), or non-transforming, like β_2 microglobulin and insulin growth factor-I (IGF-I). Cytokines like IL-1 stimulate resorption, as do epidermal growth factor and platelet derived growth factor.

Hormones such as glucocorticoids and sex hormones also play in significant role in regulating bone. Glucocorticoids seem to have a dual role on bone formation. Tissue culture experiments have shown that physiologic concentrations of glucocorticoids can increase collagen synthesis, whereas prolonged administration can inhibit bone formation.

1. 3. BIOCHEMICAL MARKERS OF BONE TURNOVER

The methods used to evaluate bone turnover can be divided into three categories:

1) Bone histomorphometry

2) Calcium kinetics and balance studies, and,

3) Biochemical markers of bone turnover.

Bone turnover can be precisely assessed by bone biopsy and histomorphometry. However, bone biopsy is not part of the routine evaluation of the osteoporotic patient. This is so, because it is an invasive procedure and requires specialized personnel and laboratory facilities for evaluation.

Calcium kinetics and balance studies require the administration of radioactive material and long periods of observation, which make them unsuitable for typical outpatient studies.

Biochemical tests performed on blood and urine samples can reliably mirror the ongoing remodelling process. These markers measure the enzymatic activities produced by bone cells, or bone matrix components that pass into the circulation during bone formation or resorption and can be listed as follows:

Markers of Bone Formation

- Serum alkaline phosphatase
- Serum osteocalcin
- Serum carboxy terminal propeptide of type I procollagen
- Urinary non-dialyzable hydroxyproline

Markers of Bone Resorption

- Urinary pyridinium cross links
- Urinary total and dialyzable hydroxyproline
- Urinary hydroxylysine glycosides
- Serum tartrate resistant acid phosphatase

There are markers that can be measured to gauge bone mineralization, like bone sialoprotein. These are however still at an experimental stage.

The biochemical markers are based on the measurement of the enzymatic activities produced in the blood or urine by bone cells or bone matrix components. These enzymatic activities pass into the blood or urine during processes of bone formation or resorption. Although the list of bone turnover markers implies that each parameter is of either bone formation or resorption, this implication should not be assumed very strictly, because of the strict coupling between bone formation and resorption. Whenever bone turnover is increased, both processes are accelerated. It is therefore difficult to establish purely on a clinical basis, whether any one biochemical marker is purely an indicator of any one phase of bone remodelling. In most cases each biochemical marker has to be considered as being predominantly associated with either bone formation or resorption.

1. 3. 1. Markers of Bone Formation

Alkaline Phosphatase

Alkaline phosphatase is an enzyme localized in the membrane of osteoblasts which is released into the circulation by an unclear mechanism. Three different genes encode for different tissue specific iso-enzymes.

1) Placental,
2) Intestinal, and,
3) Bone-liver-kidney isoform.

The function of alkaline phosphatase in bone biology is not entirely clear. Currently there are two hypotheses that postulate the role of alkaline phosphatase:

a) Alkaline phosphatase releases inorganic phosphate into the matrix and thereby promotes mineralization of bone, or,
b) Alkaline phosphatase indirectly stimulates hydroxyapatite crystal formation by cleavage of pyrophosphate, an inhibitor of mineralization.

Alkaline phosphatase is a valid marker of bone turnover. The asssumption that its activity reflects mainly bone formation than resorption is based on the fact that the enzyme is expressed by osteoblasts, cells related to bone formation. A correlation between the activity of alkaline phosphatase and histologic parameters has been demonstrated, but only in diseases characterized by extremely high bone turnover like Paget's disease and primary hyperparathyroidism. Unfortunately clear correlations have not been demonstrated in conditions associated with a relatively narrow range of bone turnover, such as postmenopausal osteoporosis associated with low to normal bone turnover.

The activity of alkaline phosphatase is related to age and sex. This is confirmed by elevated levels of the enzyme in actively growing children as compared to adults. This is believed to be due to bone isoform. Between the second and fourth decades, alkaline phosphatase is higher in males than females and this trend is reversed between the fifth and sixth decades.

The diagnostic value of alkaline phosphatase is maximum in Paget's disease of bone, even outweighing osteocalcin. However, increased levels of alkaline phosphatase may also be seen in vitamin D deficiency, X-linked hypophosphataemic rickets, hyperthyroidism and in patients treated with gold salts, non steroidal anti-inflammatory agents, allopurinol and oral hypoglycaemic agents.

Osteocalcin

Discussed in section 1.2.2.

Serum Carboxy Terminal Propeptide of Type I Procollagen (PICP)

Once the osteoblast has synthesized procollagen α, part of the non-collagenous extension peptides are cleaved from the carboxy and amino terminals prior to fibril formation. Both peptides enter the circulation and some of the amino terminal propeptide gets incorporated into the matrix and is released during bone resorption. The carboxy terminal propeptide on the other hand appears to be proportional directly

to bone matrix synthesis, and is therefore potentially a specific marker for bone formation. A correlation between PICP and histological bone formation has been demonstrated in patients with vertebral osteoporosis (Parfitt et al 1987). One drawback that limits the specificity of PICP as a marker is that it is also produced by other tissues. Furthermore, most of the circulating PICP is taken up by the endothelial cells of the liver.

PICP is a trimeric glycoprotein, with a molecular weight of 100 kD. PICP like osteocalcin, is low in children with deficient growth, and, increases during treatment with growth hormone, suggesting a close correlation with bone growth. Like alkaline phosphatase, it is elevated in Paget's disease of bone. Correlation of PICP with urinary hydroxyproline has also been demonstrated in Paget's disease. Hassager et al (1991) demonstrated good correlations between serum PICP and histomorphometric and biochemical parameters of bone. The observation that a single dose of prednisolone can decrease serum PICP, but not urinary hydroxyproline, indicates that this marker is an extremely promising index for bone formation.

1. 3. 2. Markers of Bone Resorption

Urinary hydroxyproline

When procollagen α is being processed, one of the steps in post-translation involves the hydroxylation of proline and lysine residues. This step is essential for the protein to acquire its characteristic helical shape. Hydroxyproline is therefore found almost exclusively in collagen, although a portion can be found in other proteins like the C1q fraction of complement. This fact limits the specificity of this marker as an index of bone turnover. Collagen degradation results in the release of free hydroxyproline into the circulation. This cannot be reutilized for synthesis and therefore is filtered by the kidney into the urine. The reabsorbed amino acid gets catabolized by the liver.

Total hydroxyproline in the urine consists of the dialyzable fraction (90%) and the non-dialyzable fraction (10%). The former seems to originate from collagen degradation, and represents the main bone resorption marker. The latter derives from newly

synthesized collagen fragments that are not incorporated into the matrix. It is reflective of bone formation.

The fractionation of hydroxyproline by dialysis can theoretically provide information on both, bone formation and resorption process, but this methodology is time consuming and poorly reproducible. For most clinical uses therefore, measurement of total hydroxyproline is widely used as a marker of bone resorption. Since urinary hydroxyproline is dependent directly on glomerular filtration, the results are usually corrected by creatinine excretion or clearance. It is important to remember that the excretion of hydroxyproline is heavily dependent on dietary collagen. The patients therefore have to strictly follow a collagen-free diet at least two days before the test. Hydroxyproline can be measured either from a 24 hour urine sample or a 2 hour sample, after an overnight fast, corrected for creatinine.

Urinary hydroxyproline like serum osteocalcin and alkaline phosphatase follows age and sex related changes.

Urinary Pyridinium Cross-Links

Covalent cross links are formed in the helical chains to stabilize the collagen fibrils. These cross links are aldehyde bonds between amino acid residues, mostly lysine, hydroxylysine and histidine. In bone the cross linking involves lysine and hydroxylysine, whereas in skin it is histidine. This tissue specificity forms the basis for the use of collagen cross links as markers of bone turnover.

The two major cross links in the matrix of bone and cartilage are hydroxy lysylpyridinoline and lysylpyridinoline. They are also known as pyridinoline (Pyr) and deoxy-pyridinoline (DPyr) respectively. Pyr is distributed widely in the type I collagen of bone and in type II collagen of cartilage, and in smaller amounts in other connective tissues except skin. DPyr on the other hand has been found in large amounts exclusively in bone. Both collagen crosslink metabolites pass into the circulation after collagen degradation, and are excreted as such in the urine, since they are not catabolized any further. Urinary concentrations of collagen crosslinks therefore

provide a direct measurement of bone resorption, specially the measurement of DPyr. Following bone resorption by osteoclasts, the cross links are excreted in the urine in peptide bound form (60%) and in free form (40%). The total amount can be measured by fluorimetry after reversed phase HPLC of a cellulose bound extract of hydrolyzed urine (Black et al 1988). In patients with vertebral osteoporosis, the urinary cross links levels, specially DPyr are correlated with bone turnover measured by calcium kinetics (Eastell et al 1990) and bone histomorphometry (Delmas et al 1991).

Levels of both Pyr and DPyr are increased in Paget's disease of bone, primary hyperparathyroidism, hyperthyroidism and in patients with malignant hypercalcaemia. Elevated levels have also been noted in osteoarthritis and rheumatoid arthritis. Children as compared to adults, and postmenopausal women as compared to premenopausal women have higher levels of urinary cross links.

The levels of urinary cross links have several potential advantages over hydroxyproline:

1) They are relatively specific for bone turnover,
2) They do not appear to be metabolized in vivo prior to their urinary excretion,
3) The absence of intestinal absorption of Pyr and DPyr contained in gelatin allows to collect urine without any food restriction.

A circadian rhythm has been observed in the urinary excretion of Pyr and DPyr, with peak levels observed at night and a nadir in the afternoon. Measurements should therefore be performed on a 24 hour sample. The circadian rhythm makes the analysis of a two hour sample untenable. Alternatively, a short urinary sampling may be a better index of subtle changes of bone turnover, because of the good specificity of this marker.

So far all the data have been obtained using HPLC to assay the cross links. More convenient methods are required for a broader clinical use of this marker. Immunoassay and ELISA are currently being developed. The most promising use of

the urinary cross links is in screening osteoporotic patients and for the prediction of the risk of osteoporotic fractures.

Plasma Tartrate Resistant Acid Phosphatase (TRAP)

The enzymatic properties of acid phosphatase are similar to that of alkaline phosphatase, except that it's optimum pH is acidic. The activity of this enzyme is in various tissues, including bone, platelets, spleen, prostate and erythrocytes. The bone isoenzyme of acid phosphatase is only expressed by osteoclasts, and therefore is specific for bone resorption. An added advantage is that this bone isoenzyme can be isolated from the other isoenzyme because it is resistant to tartrate. Indeed, this is the only form of isoenzyme that is resistant to tartrate, and this characteristic feature is employed to differentiate this form from the prostatic component, because the latter is sensitive.

High levels of tartrate resistant acid phosphatase have been observed in patients with conditions with high bone resorption like Paget's disease of bone, primary hyperparathyroidism, glucocorticoid treatment and hyperthyroidism. A good correlation has been observed between TRAP and urinary hydroxyproline in oophorectomized women, in the first few years after surgery (Stepan et al 1987). Unfortunately correlations with histomorphometric parameters are still scanty. Refinement of the assay possibly utilizing more immunological techniques are required to utilize the potential of this marker fully.

Urinary Hydroxylysine Glycosides

One of the steps in the posttranslational processing of procollagen α is the hydroxylation of lysine and proline residues. Therefore the excretion of hydroxylysine residues also reflects breakdown of bone matrix. Since hydroxylysine glycosides are excreted entirely into the urine, the measurement of this amino acid provides a more selective index of bone resorption as compared to hydroxyproline.

There are two predominant types of hydroxylysine glycoside residues. Beta-1-galactosyl hydroxylysine (GHL) is prevalently a product of bone collagen, whereas, alpha-1,2-glycosyl-galactosyl hydroxylysine (GGHL) is more specifically related to skin collagen. The GHL/GGHL ratio provides a good index of the metabolic activity of either tissue; increasing in Paget's disease of bone and decreasing in patients with extensive burns.

One of the difficulties faced in clinical practice which limits the use of this marker in routine clinical practice is the method of its assay. High performance liquid chromatography is required to separate the different forms, contributing to its limitations.

1. 4. OSTEOPOROSIS IN MEN

The mineral content of bone and the architectural structure determine the mechanical properties of bone. The reduction of bone mass is an inevitable accompaniment of ageing, and this causes an increased propensity to fracture. The major sites sustaining osteoporotic fractures are the femoral neck, vertebral body and the forearm. There is a slight difference in the prevalence of fracture rates between men and women, which possibly reflects the risk of falling; there being an increase in falls in middle aged women as compared to middle aged men. The precise incidence of vertebral fractures is difficult to ascertain, as only 35% of such fractures involve medical consultation (Cooper 1992).

There is an increase in the number of men presenting with fractures due to osteoporosis. This is possibly due to a shift in the demographic trend towards an ageing population and a doubling of the age-specific incidence of fractures over the last three decades (Boyce and Vessey, 1985, Obrant et al 1989).

1. 4. 1. Peak Bone Mass and Factors Affecting It

The risk of fractures is to a large extent determined by peak bone mass, which is the age at which bone loss starts and the rate at which it proceeds. The peak bone mass is higher in men than women, whereas, bone density at maturity is similar in both sexes (Bonjour et al 1991). Factors influencing peak bone mass include race, heredity, calcium intake during childhood and adolescence, hormonal factors, physical activity during childhood and adolescence. Of these, genetic factors account for almost 80% of the variance in peak bone mass. Physical mobility is also an important factor. Bone density is higher in children and adults of both sexes who are more active than sedentary individuals. The effect of calcium intake on peak bone mass has been studied in great detail, including a number of studies involving twins. The dietary requirement for calcium increases during the growth spurt in puberty, and several studies have indicated that a high calcium intake in this period has a beneficial effect on peak bone mass.

Factors having an adverse effect on peak bone mass include smoking, alcohol consumption, a sedentary lifestyle and low calcium intake. The adverse effect is seen in both men and women.

1. 4. .2. Bone Loss

Riggs and Melton (1986) have shown that trabecular bone loss starts in both sexes at about the age of 35, whereas cortical bone loss starts at the age of 45. Men lose 15-45% of trabecular bone and 5-15% of cortical bone with age. In comparison, women lose 35-50% trabecular bone and 25-50% of cortical bone (Riggs and Melton, 1986). Francis et al (1989) observed that age related bone loss in men is characterized by a reduction in bone formation rather than an increase in bone resorption; besides which, in men there is a greater preservation of trabecular architecture during bone loss as compared to women. Factors implicated in the pathogenesis of age related bone loss in men include heredity, hormonal factors, physical inactivity, alcohol consumption, smoking and reduced calcium intake.

Rate of bone loss to a large extent is determined by genetic factors. Studies in twins have demonstrated that there is a greater concordance between the loss of bone and biochemical markers of bone in female monozygotic than dizygotic twins.

Bone loss in women increases significantly during menopause due to a marked reduction in the circulating levels of oestradiol and progesterone. In men a fall in testosterone levels has been observed, though this is seen as late as 70 years of age. No endocrine abnormality, similar to menopause in females has been demonstrated in middle aged men. A weak correlation is known to exist between free testosterone index (ratio of serum testosterone to sex hormone binding globulin) and bone mineral density of the distal and ultra-distal radius (Kelly et al 1990).

Alcohol consumption in excess and smoking tend to depress osteoblastic activity by a direct toxic effect and thereby reduce bone formation in both sexes. Francis et al (1992) demonstrated a weak inverse relationship between alcohol consumption and low forearm bone density in males, but no relationship has been demonstrated between

smoking and bone mass. Studies looking at the relationship between calcium intake and bone mass are numerous. The results are controversial. Whereas some studies have demonstrated a negative relationship between calcium intake and bone mineral density, other studies have failed to show any relationship. The intake of calcium in childhood and adolescence has a positive effect on peak bone mass, whereas, the same cannot be said for calcium intake after 30 years of age. However, as calcium absorption decreases with advancing age in both men and women, this needs to be compensated by increasing the intake of dietary calcium. The reduction in calcium absorption can also be due to a decrease in the levels of 25-hydroxyvitamin D secondary to reduced cutaneous production and decreased absorption and hepatic metabolism.

1. 4. 3. Causes of Osteoporosis in Men

Osteoporosis in men can be primary or secondary to any cause. An underlying secondary cause of osteoporosis in men can be detected in upto 55% of men with vertebral fractures (Francis et al 1989). The major causes of secondary osteoporosis in men are steroid therapy, hypogonadism, skeletal metastases due to malignancy, multiple myeloma, gastric surgery and anticonvulsant treatment, with more than one cause being detected in about 10% of cases.

Francis et al (1989) observed that men with primary osteoporosis who have vertebral crush fractures tend to have reduced cortical and trabecular bone mass, a decrease in the number of trabeculae and an increase in the biochemical markers of bone turnover compared with age matched controls. The serum sex steroid concentrations in individuals presenting with vertebral crush fractures due to primary ostepoporosis were however similar to age-matched control subjects (Francis et al 1989). These individuals also had a reduction in their 1,25 $(OH)_2D_3$ concentrations leading to a decrease in the absorption of calcium. The increase in bone resorption in this group of patients was minimal and was probably not enough to account for the observed reduction in serum levels of $1,25(OH)_2D_3$.

Cooper et al (1988) in a case control study of femoral neck fractures indicated that physical inactivity and low calcium intake were significant factors, although the effect of calcium intake disappeared when allowance were made for confounding variables.

1. 4. 4. Hypogonadism Causing Osteoporosis in Males

Hypogonadism is a well established cause of osteoporosis in males. It is found in about 25-30% of men with vertebral crush fractures. The causes of hypogonadism in males leading to osteoporosis include Klinefelter's syndrome, idiopathic hypogonadotrophic hypogonadism, hyperprolactinaemia, haemochromatosis and primary testicular failure (Jackson and Kleerkoper, 1990). There is a reduction in both trabecular and cortical bone mass in osteoporosis due to hypogonadism and histological studies have demonstrated an increase in bone resorption and a decrease in mineralization (Francis et al 1986). The pathogenesis of bone loss remains unclear although the various factors implicated are androgen/oestrogen deficiency, malabsorption of calcium, low levels of $1,25(OH)_2D_3$, and a reduction in the levels of circulating calcitonin. These are commonly associated with hypogonadal osteoporosis. Testosterone can reverse these changes and can also decrease bone resorption and stimulate bone mineralization (Francis et al 1986).

The diagnosis of hypogonadism is not always clinically apparent in men with osteoporosis, therefore routine measurement of serum testosterone and sex hormone levels may be worthwhile.

1. 4. .5. Prevention of Osteoporosis in Men

Treatment of established primary osteoporosis in men is largely empirical. Therefore emphasis should be placed on prevention. Preventive measures should be aimed at achieving peak bone mass, reducing bone loss and decreasing the risk of falls, particularly in elderly persons.

Ideally, increasing the intake of dietary calcium and exercises should be instituted during childhood and adolescence. Maintaining physical activity can decrease the risk of falls. Although the role of dietary calcium in the prevention of bone loss remains controversial, it is possibly prudent to maintain a diet containing at least 700 mg of calcium daily. Moderation of alcohol and tobacco consumption is certainly beneficial as both have an inhibitory effect on osteoblast function. It is important to ensure that elderly men are not vitamin D deficient. This would prevent the decline in calcium absorption and decrease bone loss. It is important therefore to rule out osteomalacia. Therapeutic vitamin D supplementation should be considered in housebound or immobile individuals.

Another important consideration should be the exclusion of secondary osteoporosis. As causes of secondary osteoporosis may not always be apparent clinically, it is appropriate to carry out routine investigations aimed at excluding causes of secondary osteoporosis. It is important not to forget inflammatory conditions as causes of osteoporosis. Many connective tissue diseases can cause osteoporosis. Patients with rheumatoid arthritis develop severe disability as a result of the complications of osteoporosis. Steroids are a common therapeutic measure in patients with connective tissue diseases, thereby accelerating the development of osteoporosis in these patients.

Effective treatment of the connective tissue disease, with gradual reduction of steroids may help to prevent or reduce the severity of the osteoporosis.

1. 5. OSTEOPOROSIS IN INFLAMMATORY ARTHRITIDES

The association of osteoporosis with inflammatory conditions is well recognized.

Patients with rheumatoid arthritis have osteoporosis in two forms:

(1) Juxta-articular osteoporosis which occurs around inflamed joints and appears within weeks of onset. This is also a characteristic feature of early disease, and;

(2) Generalized axial and appendicular osteopaenia. This contributes to the increased risk for fractures of the femoral neck and the vertebral body.

Inverse correlations have been demonstrated between disease activity in rheumatoid arthritis and the development of osteopaenia. This is accelerated if the patient is on steroids for disease control. Numerous difficulties are faced during rehabilitation of patients of rheumatoid arthritis with vertebral and hip fractures. It is therefore important to recognize this problem and institute proper preventive measures.

Osteopaenia is seen not only in patients with rheumatoid arthritis, but also in many connective tissue diseases. It is thought that these effects are possibly mediated by cytokines and growth factors. The presence of cytokines in bone has been described by many researchers. In some but not all cases there is evidence that these factors may be synthesized by osteoblast like cells. It is also possible that some of these biologically active materials may be derived from other cellular sources, e.g. platelets, lymphocytes or macrophages.

Rheumatoid arthritis is characterised by low bone formation (Ekenstam et al 1986) and increased bone resorption (Black et al 1989, Seibel et al 1989).

Numerous studies have demonstrated low bone mineral density in the lumbar spine and femoral neck in rheumatoid arthritis but the relationship between bone mineral density and fractures in the vertebrae is not very clear (Spector et al 1993).

Low testosterone levels have also been demonstrated in patients with rheumatoid arthritis (Spector et al 1988). Sex hormones are known to affect the bone mass and

immune response in cases of inflammatory arthritis. The implications of the low androgenic hormone in inflammatory conditions is not well established.

There are differences between the osteoporotic process in involutional osteoporosis and that seen in inflammatory conditions. The relationship between the cytokines, biochemical markers of bone turnover, bone mineral density and vertebral fractures is not well established in inflammatory arthritides and this study has been performed with the aim of answering a few questions about the pathophysiology of osteoporosis that is associated with ankylosing spondylitis.

This knowledge about multiple possible regulatory mechanisms within bone should aid the understanding of physiological bone remodelling and offer potential explanations for changes in bone turnover seen in a variety of disease states.

1. 6. OSTEOPOROSIS IN ANKYLOSING SPONDYLITIS

One of the most intriguing features of ankylosing spondylitis (AS) is the co-existence of excessive calcification and bone formation in extra-osseous (spinal) tissues along with osteoporosis in the axial skeleton. Most studies looking at the radiological changes in AS have focussed on the enthesopathy and the florid syndesmophytosis seen in this condition, whereas relatively few studies in comparison have looked at the development of osteoporosis in AS. Researchers studying the latter have described contradictory findings with regard to the development of osteoporosis. Some authors feel that the osteoporosis develops late in the course of the disease and is related to the relative immobility of the spine, while others have described its development early in the disease and feel it is due to the associated inflammation.

Osteoporosis in AS was noted as early as 1877 when Fagge, in an autopsy report described the excessive softness of vertebrae in a patient with AS. This was later confirmed by Buckley in 1932 who felt that the osteoporosis in AS developed early in the disease. He stated that, " In ankylosing spondylitis the earliest change to be detected roentgenologically is rarefaction of the vertebral bodies," and that, "the rarefaction or osteoporosis of the vertebral bodies is responsible for the curvatures which are apt to develop." Polley, (1955) on the other hand stated that osteoporosis in AS developed as a late manifestation of the disease.

Studies looking at the development of fractures in patients with AS as a consequence of the underlying osteoporosis are also contradictory with regard to the prevalence of fractures noted. Wilkinson et al (1958), Hunter et al (1978 1983) and Hanson et al (1971) found the prevalence of vertebral fractures to be very low. They also found that the fractures were related to spinal trauma and correlated directly with the duration of disease. Dilsen (1964) and Ralston et al (1990) found the prevalence of fractures to be very high. They also observed that the development of fractures in their patients correlated directly with disease duration. Dilsen described his findings in a series of 97 patients with AS. He graded the osteoporosis noted into three categories and

correlated the radiological findings to age, sex and the duration of disease. He reported 11 lumbar, 22 thoracic and 2 cervical crush fractures in these 97 patients, a prevalence of 29%. Although he found a significantly positive correlation between the osteoporosis and disease duration, he could not demonstrate a correlation between the presence of osteoporosis and the age of the subject. Ralston et al (1990) also found a high prevalence of fractures and pointed out that the development of vertebral fractures in their group of patients was spontaneous and *not related to trauma* unlike that seen in the previous studies. Their conclusion therefore was that it was the osteoporosis that was responsible for the development of fractures in these patients.

Although Wilkinson et al and Hanson et al found a direct correlation between the osteoporosis and disease duration, they felt that the low incidence of fractures in their patients was possibly due to the fact that the syndesmophytes protected the rigid spondylitic spine against fractures. Ralston et al on the other hand felt that it was the immobilty of the spine due to the syndesmophytes that actually predisposed the vertebrae to fracture.

Cooper et al (1994) attempted to look at the site specific fracture risk in patients with AS. They found a "significant increase in the risk of clinically diagnosed thoracolumbar vertebral compression fractures in patients with AS but no elevation in the risk of limb fractures." They also noted a higher fracture risk in male patients with AS as compared to female patients. It is interesting that as compared to fractures in the vertebrae the risk of fractures in the appendicular skeleton was not elevated even with long standing disease. This is consistent with the findings of normal bone mineral density (BMD) of the appendicular skeleton in patients with AS (Will et al 1989, Devogelaer et al 1992, Mullaji et al 1994, Ralston et al 1990). The risk of vertebral fractures seems to increase with the duration and severity of disease.

Not all studies looking at osteoporosis in AS however have commented on vertebral fractures. Spencer et al in 1979 found that 30% of their patients with AS had vertebral

osteoporosis. Their study however does not discuss the prevalence of vertebral fractures. With the development of techniques measuring bone mineral content and density in individuals, the diagnostic sensitivity for osteoporosis has increased greatly. No longer do researchers have to rely on insensitive methods like the plain radiograph. It is however important to realize that densitometric study of AS is limited by the new bone formation seen in this condition, and as a consequence yields inconsistent findings.

Reid et al (1986) reported high BMD using dual photon absorptiometry (DPA) in the lumbar spine in patients with AS, whereas, Will et al (1989) using DPA and Mitra et al (1994) using dual energy x-ray absorptiometry (DXA) reported low lumbar bone density in their patients. It is noteworthy that patients in the former study had advanced radiological changes in their spine while patients in the latter two studies had early disease, with relatively normal lumbar spines.

Devogelaer et al (1992), Mullaji et al (1994) and Lanyi et al (1993) have looked at the differences in BMD, using DXA, in patients with AS with minimal lumbar spine involvement radiologically and in patients with syndesmophytes in their spine. Their studies comprised of 70, 33 and 44 subjects respectively. All three studies confirm a decrease in the lumbar BMD in patients with minimal lumbar spine disease compared with normal controls. In contrast, Devogelaer et al and Lanyi et al found no significant difference in the BMD of the patients with advanced radiological changes compared with the controls. Mullaji et al on the other hand found a significant increase in the lumbar spine BMD in patients of AS with syndesmophytes compared to controls.

Although a decrease in the BMD of the hip has been described (Will et al 1989, Mitra et al 1994, Mullji et al 1994) a similar reduction in BMD of the appendicular skeleton (non-dominant radius and carpus) has not been described. On the contrary, as mentioned above, Devogelaer et al (1992), Will et al (1989), Mullaji et al (1994) and Ralston et al (1990) found the bone density to be normal in the appendicular skeleton

(forearm and carpus) of patients with either mild or advanced AS as compared to controls.

It is still unclear as to whether the development of osteoporosis in AS occurs early or late in the disease. There is as yet no comprehensive evidence to support either. With the use of sophisticated techniques to determine BMD, it has been established that low bone mass is definitely present in patients with "mild" AS who have normal mobility of the spine and whose vertebrae do not have the new bone formation so characteristic of AS. The use of QCT has documented the presence of trabecular osteoporosis in AS (Devogelaer et al 1992). Will et al (1989) suggested that the osteoporosis in AS was a primary pathological event. They postulated this due to the low BMD seen in the "normal" lumbar vertebrae of their patients coupled with the presence of osteoporosis in the femoral neck. Their study however failed to address their basic question as to whether the bone loss in AS was a primary pathological event, perhaps mediated by interleukin-1 (IL-1) and tumour necrosis factor (TNF). These two cytokines are responsible for subchondral bone resorption. The fact that the TNFα and TNFβ genes lie in tandem within the MHC between the class IB genes and the class III genes is relevant to the investigation of bone disease in AS. Researchers attempting to answer this question have variously looked at cytokine levels, sex hormones and biochemical markers of bone metabolism in AS.

The pathogenetic mechanism for the development of AS has long been debated. The lack of comprehensive knowledge about this adds to the difficulty in understanding the pathophysiology of the clinical features seen in this condition. It is still unclear as to what causes the osteoporosis seen in AS, significant enough perhaps to cause vertebral fractures and therefore the postural deformity that is responsible for the morbidity associated with this condition. As immobility can cause osteoporosis, it is possible that this may lead to vertebral osteoporosis in patients with radiologically advanced spinal disease. This explanation however does not suffice for the osteoporosis seen in the hip, and in spines that have no syndesmophytes. Researchers responsible for the latter observation have been unable to give a satisfactory explanation for the development of

osteoporosis. In an effort to answer this question scientists have tried to look for possible contributory factors.

Houssiau et al in1988 showed that the levels of interleukin-6 (IL-6), which is usually induced by IL-1 and TNF, were elevated in the synovial fluid of patients with AS, but not in their serum. In contrast Bilgic et al (1994) showed an abnormal increase in the serum IL-6 levels in 86% of patients with AS. They however failed to show a correlation of IL-6 with either the clinical variables or acute phase reactants [erythrocyte sedimentation rate (ESR), plasma viscosity and C-reactive protein (CRP)]. The significance of the elevated levels of IL-6 in patients of AS remains unknown. There is no other published report on the role of cytokines in AS.

Serum and free testosterone levels in males with rheumatoid arthritis seem to be lower than that seen in male patients with AS or normal healthy male controls (Spector et al 1988). It has been suggested that patients with AS may have a testosterone/oestrogen imbalance (Chevallard et al 1987, Borbas et al 1986, Dougados et al 1986), and that high testosterone levels may be a risk factor for the disease. The sample size however in the latter three studies was small. Spector et al (1988) however did not notice any difference in total and free testosterone levels or in sex hormone binding globulin between the AS patients and controls. Tapia-Serrano et al (1991) reported a lower testicular reserve of testosterone in men with AS.

Attention has also focussed on biochemical markers of bone metabolism in patients of AS. Reid et al (1986) found the total body calcium to be low in their subjects, with the reduction being more pronounced in their group of female patients. Franck et al (1993) observed that serum osteocalcin levels were significantly reduced in their series of 38 patients but the vitamin D levels were slightly but not significantly higher as compared to the controls. They also found a significant elevation in the levels of alkaline phosphatase in these patients, but did not find a significant correlation between alkaline phosphatase and osteocalcin.

The pathophysiology of osteoporosis still remains unclear. This study endeavours to answer a few questions that have been raised regarding the development of osteoporosis and fractures due to osteoporosis in patients with AS in the axial skeleton.

1. 7. VERTEBRAL FRACTURES AND THEIR RELEVANCE IN ANKYLOSING SPONDYLITIS

1. 7. 1. What is a Vertebral Fracture?

One of the major difficulties that osteoporosis researchers face, is determining the presence or absence of vertebral fractures. Vertebral fractures are an inherent part of osteoporosis and are a cause of severe disability when extensive. Unfortunately fractures may go undetected at times as they may be symptomless. The major clinical features are, pain, kyphosis and loss of height. Their presence also increases the risk of further fractures. It is important to quantify and characterise fractures to be able to treat the patient optimally.

Vertebral morphometry is an objective and reproducible method for assessing vertebral deformity and involves the measurement of vertebral dimensions and comparing the ratio of these heights with reference values derived from individuals without deformity. This is of use in determining the prevalence and incidence of vertebral fractures.

The characteristic deformities seen in fractures due to osteoporosis are anterior wedge fractures, biconcave deformities (central compression fractures) and crush fractures, with collapse of the entire vertebra. Vertebral deformities can occur not only due to osteoporosis but also due to causes such as trauma. In addition, there are conditions such as Scheuermann's disease and epiphysial dysplasia that can cause changes in the vertebral bodies that mimic deformities. All conditions causing axial osteoporosis can cause vertebral fractures. In assessing vertebral fractures attention needs to be paid to these causes of deformities.

It is not difficult to diagnose an obvious vertebral fracture. The difficulty arises when the change in vertebral shape is more subtle. The more subtle the radiologic criteria used, the greater the prevalence of fractures and greater the possibility that the fracture is of no clinical significance. Statistically the number of false positives increase when the criteria are subtle. Many researchers define a vertebral fracture as a decrease in the anterior, posterior or central height of a vertebra by 15-25%. The percentage decrease

in vertebral height has to be variable, as a fixed percentage decrease can result in a change in the prevalence of fractures, depending on the vertebral level. Many vertebral deformities are not clinically significant events. It is possible that some of these represent gradual shape changes that result from internal changes in bone architecture. It has been seen however that abnormal vertebral morphometry resembling midthoracic vertebral fractures can be seen even in the absence of osteopaenia.

The distinction between vertebral deformity and vertebral fractures is important for epidemiological studies. The prevalence of vertebral deformity is much greater and includes vertebral fracture. The symptomatic event due to a vertebral fracture is quite small and associated with limited morbidity, whereas, the consequences of deformities, such as progressive thoracic kyphosis, loss of stature, chronic pain and altered body image are significant and lead to long term morbidity, affecting quality of life.

1. 7. 2. Quantification of vertebral fractures by morphometry

In contrast to fractures of the appendicular skeleton, the epidemiology of vertebral fractures is not well documented for a number of reasons. The presence or absence of an appendicular fracture is usually obvious whereas the presence of a vertebral fracture depends upon the assessment of changes in vertebral shape. Several techniques have been developed to quantitate vertebral deformities, usually on the basis of assessing the heights of vertebral bodies (McCloskey et al 1993, Eastell et al 1991, Melton et al 1989, Riggs et al 1982, Raymakers et al 1990, Kleerekoper et al 1984, Gallagher et al 1988, Hedlund et al 1988, Minne et al 1988, Black et al 1991).

The methods can be semiquantitative (Kleerekoper et al 1984) in which a score is given to each vertebra according to its shape on visual inspection, or quantitative. The quantitative methods can be based on the ratio of the height measurements of each vertebra (Hedlund et al 1988, Riggs et al 1982), or they may be calculated by comparing the entire set of vertebral heights to normative data from the general population (Minne et al 1988, Gallagher et al 1988). Eastell et al (1991), Melton et al (1989) and McCloskey et al (1993), described morphological techniques which did not utilize absolute vertebral heights so that errors associated with positioning and

projection are eliminated. The method described by McCloskey et al is fairly robust and has a much lower false positive rate.

The advantage of all these methods is that they reduce bias in the interpretation of radiographs, because of their objectivity. Additionally, they detect minor vertebral deformities which is advantageous in osteoporosis research. As mentioned above, minor vertebral deformities may not have any clinical relevance, which can be a disadvantage. The assessment of specificity and sensitivity has been limited by the lack of uniformity in the description of normal and deformed vertebrae. As there is no standardization of these methods, this results in differences observed in the apparent prevalence of vertebral fractures reported in the community. Clinical trials are also confounded by the wide difference noted in the incidence of new vertebral fractures in postmenopausal osteoporosis.

1. 7. 3. Relevance of vertebral fractures in Ankylosing Spondylitis

Studies looking at fractures in advanced AS give conflicting results. Hanson et al (1971) suggested that the prevalence of fractures in advanced AS was low because the syndesmophytes had a protective effect on the spine, and therefore prevent the development of fractures. They implicated trauma as the cause of fractures in their group of patients. Ralston et al (1990) on the other hand observed a high prevalence of fractures in their patients with advanced AS and since none of their patients had a history of trauma, they suggested that the fractures could be spontaneous as a result of osteoporosis. Donnelly et al (1994) did not find a significant reduction in the bone mineral density of the spine or hip in patients with AS who had vertebral fractures compared with those without. The study described in chapter three aims to determine the prevalence and distribution of vertebral deformties in AS, and the relationship between deformities with bone mineral density of the lumbar spine and femoral neck.

1. 8. RADIOGRAPHIC DIAGNOSIS OF OSTEOPOROSIS

Although bone loss and osteopaenia may be evident on ordinary radiographs, it is one of the most insensitive methods of diagnosis of osteoporosis. The development of osteoporosis leads to a reduction in bone strength. Bone strength as such depends on bone mineral density (BMD), bone structure and size, material properties of the bone matrix and the ability to heal microfractures. Of the methods available, bone mineral density measurements are the most important determinants of bone fragility. Direct estimation of BMD is therefore the most useful measurement to make in the assessment of an individual's fracture risk. This raise the fundamental question, what area should be measured to predict the risk of fracture?

Bones that are composed of a high proportion of trabecular bone such as the vertebral body, the neck of femur and the distal radius are obviously at greater risk for the development of fractures than other sites. BMD measurement of these sites is the common practice. The prediction of fractures is site specific, in that, BMD measurement of the lumbar spine best predicts vertebral fractures and likewise, BMD measurement of the proximal femur best predicts hip fractures (Cummings et al 1993).

1. 8. 1. Techniques used to measure bone mineral density

Over the years several techniques have been used to measure bone mineral density. They are summarized in table 1.8.1.a. and are described briefly below.

Table 1. 8. 1a. *Techniques currently in use to measure bone mineral density*

Technique	Precision (%)	AccuracyError (%)	Radiation Dose (μSv)
SPA	1-2	4-6	<1
DPA			
Lumbar Spine	2-4	5-10	5
Proximal Femur	3-5	5-10	3
DXA			
Lumbar Spine (AP)	1	4-8	1
Lumbar Spine (Lat)	2-3	5-10	3
Proximal Femur	1-2	4-8	1
Total Body	1	1-2	3
QCT			
Single Energy QCT	2-4	5-15	50
Dual Energy QCT	4-6	3-10	100
Peripheral QCT	0.5-1	2-8	<2
Ultrasound (US)			
Broadband US attenuation (calcaneus)	1.6-3.8	NA	0
Speed of Sound (calcaneus)	0.3-1.9	NA	0
Speed of Sound (patella)	<2	NA	0

SPA - Single Photon Absorptiometry,

DPA - Dual Photon Absorptiometry,

DXA - Dual Energy X-ray Absorptiometry.

QCT - Quantitative computed tomography.

Single Photon Absorptiometry (SPA)

SPA is a quantitative method used for measuring peripheral BMD. The sites are usually the distal radius and the calcaneus. In this method a highly collimated photon beam from a radionuclide source (usually iodine-125 [I^{125}]) is used to measure photon attenuation. The level of attenuation is then converted to bone mineral content in grams or area BMD in grams per centimeters squared. The extremity that is measured is usually kept under water during the measurement to keep the absorption of the soft tissue constant and to compensate for superimposition. A disadvantage of the SPA is that separate measurements of trabecular and cortical bone are not possible with this method. The commonly used site for bone densitometry by SPA is the distal third of the radius. This is composed mainly of cortical bone. The type of bone measured in this is mainly cortical. One can measure the ultradistal radius as well, which yields a trabecular to cortical bone ratio of about one, which is similar to the lumbar spine. The value of BMD measurements at the calcaneus using SPA has been somewhat controversial due to the potentially confounding relationship between BMD and body weight or exercise at this site.

SPA has proved to be a useful tool in the diagnosis of osteoporosis. Some systems using SPA are now gradually replacing the radionuclide source by an x-ray tube resulting in improved cost effectiveness and precision.

Dual Photon Absorptiometry (DPA)

The technique of SPA has a major limitation in that it cannot measure BMD at thick body sites, where the soft tissue and body composition is variable, i.e. the axial skeleton, hip or whole body. To circumvent this problem absorptiometry techniques using radiation of two different energies have been developed and studied. The technique, which was an extension of the principle of SPA is called dual photon absorptiometry. DPA uses a radionuclide source at two effective discrete energies, gadolinium-153 (Gd^{153}) with energies of 44 and 100 keV. With this method the bone and soft tissue components can be differentiated and variations in soft tissue thickness can be accounted for. BMD at sites inaccesible to SPA like the lumbar spine, proximal femur and total body is possible with DPA.

Dual Energy X - ray Absorptiometry (DXA)

In the past six years DPA has been replaced by DXA. This technique is based on the same principle as DPA but uses an x-ray tube instead of the radionuclide source. The advantage of the x-ray source is that it is more stable than the isotope source and does not require as frequent replacements. The main advantage however of an x-ray system over a radionuclide source is the increased photon flux facilitating a finer collimation of the beam to about 1.5 mm compared to 5-8 mm for the DPA. Additionally the use of an x-ray system results in shorter scan time, greater accuracy and precision, higher resolution and the lack of radionuclide decay. A strong correlation has been demonstrated between DPA and DXA which allows normative data generated by DPA to be extrapolated to DXA with appropriate offsets.

Using DXA, the areas that can be scanned include the lumbar spine, the proximal femur, the whole body, the forearm and the calcaneus. The new generation of scanners like the Hologic QDR 2000 and the Lunar Expert use fan beams and multidetector arrays instead of a pencil beam and single detector, decreasing the examination time considerably. With these machines lateral scanning of the lumbar spine is possible. The measured BMD can be artificially increased in postero-anterior measurements of the lumbar spine due to the possible presence of osteophytes, aortic calcifications, degenerative facet sclerosis, and intervertebral space narrowing in degenerative disc disease. In addition the area measured includes a substantial portion of cortical bone. With lateral measurements of the lumbar spine these problems are eliminated as it is possible to evaluate the vertebral bodies alone. Improvement has been noted in discriminating age related bone loss and in distinguishing normal from osteoporotic women with lateral DXA. The new generation of DXA scanners have rotating x-ray tubes so that problems no longer arise due to difficulty in reproducing the lateral position. The patient can now lie supine and lateral measurements can be taken after postero-anterior measurements with the patient in the same position.

Quantitative Computed Tomography (QCT)

One can measure a three dimensional true density of bone without superimposition of other tissues by QCT. The bone density can be calculated separately in trabecular and cortical compartments. In osteoporosis research the measurement of trabecular bone in the central portion of the vertebral body is of use as this effectively precludes cortical bone and extraosseous calcifications. A single energy quantative CT is usually used for routine examinations. One of the relative disadvantages of the QCT is that absorption is influenced not only by bone but also by other constituents like bone marrow fat. Bone marrow fat which increases with age interferes with the measured density, underestimating the actual bone mineral content by 15 - 20%. The clinical relevance of the fat error is small, owing to the development of age matched databases. Dual energy QCT can improve on this but at the cost of a higher radiation dose. Besides, the technical difficulty and higher cost restricts the use of dual energy QCT to research on the whole.

QCT is generally used to measure the bone mineral density of peripheral sites and the axial skeleton, but not the proximal femur.

Quantitative Ultrasound

Ultrasound transmission velocity (UTV) has been used to test the material properties of bone as early as the 1960s. The UTV or *speed of sound* (SOS) is calculated as the quotient of the transit time of an ultrasound wave through bone and the diameter of this bone, in meters per second. Bone mass and qualitative characteristics of bone contribute to the UTV. A few studies have looked at the correlation between UTV and BMD measurements, the results are however controversial.

Attenuation measurements of ultrasound are also performed. Reflection and absorption are the main components that attribute to the attenuation of the ultrasound penetrating a material. The attenuation also depends on the frequency of the ultrasound used. With slow frequency range, the attenuation is linear. With a higher frequency range, the attenuation is greater and nonlinear. In quantitative ultrasound techniques, the attenuation of ultrasound is measured using the low frequency range (200-600 kHz).

This technique is called *broadband ultrasound attenuation* (BUA). Clinical studies using the BUA measurements have mainly concentrated on the os calcis as the measurement site. Some correlation has been seen between BUA measurements and low bone density.

A main advantage of ultrasound examination is the complete absence of radiation and low cost of the equipment. However, the uncertain relationship between bone mass, elastic properties of bone and ultrasound, the influence of surrounding soft tissue, the path of the ultrasound wave when penetrating the bone, and the effect of physical activity are issues that are still unsolved in the application of ultrasound as a diagnostic tool.

1. 9. BISPHOSPHONATES IN OSTEOPOROSIS

1. 9. 1. Chemistry

Bisphosphonates are compounds characterised by two C - P bonds. The compounds are called geminal bisphosphonates if the two bonds are attached to the same carbon atom. The bisphosphonates are therefore analogous to pyrophosphate, but contain a carbon atom instead of an oxygen atom (fig 1.9.1.a).

Figure 1. 9. 1. a. *Structure of bisphosphonates: the central carbon atom, replacing oxygen in pyrophosphoric acid, distinguishes the bisphosphonate.*

The following bisphosphonates have been investigated in the context of bone disease in humans:

- Etidronate
- Clodronate
- Pamidronate
- Alendronate
- 6-amino-1-hydroxyhexylidene bisphosphonate
- Tiludronate
- Risedronate
- 3-dimethylamino-1-hydroxypropylidene bisphosphonate
- 1-hydroxy-3-methylpentylamino-propylidene bisphosphonate (BM 21.0955)
- 1-hydroxy-3-(1-pyrrolidinyl) propylidene bisphosphonate (EB 1053).

It is important to note that small changes in the structure of bisphosphonates can lead to extensive alterations in their physicochemical and biological properties. This therefore makes it mandatory for researchers to consider each bisphosphonate on its own and not extrapolate the results from one compound to others. These compounds demonstrate a strong affinity for metal ions such as calcium, magnesium and iron. They tend to bind to the crystal surface and act as crystal poisons for both growth and dissolution.

1. 9. 2. Pharmacokinetics

The bisphosphonates are absorbed, stored and excreted unaltered. The intestinal absorption is very low, between 1 - 10% of an oral dose. Intestinal absorption tends to decrease even further if the drug is given with meals, particularly milk. 20 - 60 % of the absorbed dose is localised in bone and the rest is excreted in urine (Fleisch 1988).

The plasma half life is fairly short, approximately 2 hours in humans. It enters the skeleton rapidly and get deposited mainly in areas of bone formation (alendronte tends to get deposited under osteoclasts preferentially). The half life in bone is estimated to be a year in animals. The renal clearance of bisphosphonates is high.

1. 9. 3. Effect of bisphosphonates on bone resorption

It has now been firmly established that bisphosphonates can inhibit the resorption of bone both *in vitro* and *in vivo*. The various bisphosphonates seem to have a similar effect on bone resorption, except for their potency and side effects. Their efficacy has been demonstrated in various types of osteoporosis and includes osteoporosis due to oophorectomy, castration, steroids, heparin, diet and immobilisation.

The mechanism of action of bisphosphonates by which they inhibit the resorption of bone is still unclear. It is undisputed that the main action of bisphosphonates is on the osteoclast. Once the osteoclast ingests the bisphosphonates various biochemical mechanisms may then play a role, such as decrease in lactic acid production, proton secretion, prostaglandin synthesis, inhibition of lysosomal enzymes and increase in

membrane leakiness (Fleisch 1988). Although these effects have been noted *in vitro* studies, the very fact that bisphosphonates reach concentrations as high as 1mM in the osteoclast suggests that these mechanisms are probably valid. It has also been found recently that bisphosphonates can act *in vitro* through the osteoblast by decreasing the release of their osteoclast stimulating factors (Fleisch 1993). It is possible that multiple mechanisms are operating *in vivo* to inhibit the activity of osteoclasts.

1. 9. 4. Use of Bisphosphonates in Osteoporosis

It is postulated that the progression of osteoporosis is dependent on an *imbalance* between bone formation and resorption of each bone remodelling unit, called the bone multicellular unit (BMU). The *rate* of bone remodelling also plays a role in the development of osteoporosis. High turnover osteoporosis (active osteoporosis) is characterized by an increase in the number of BMUs and an increase in bone resorption, whereas low turnover osteoporosis (inactive osteoporosis) is primarily due to a decrease in the number of BMUs and a decrease in bone formation.

It has been firmly established that bisphosphonates decrease bone turnover, as assessed by measuring biochemical markers of bone turnover and by histomorphometric studies measuring the BMUs. It is not clear whether bisphosphonates improve the balance at the level of each BMU. Theoretically an improvement in bone mass at the BMUS should occur by decreasing the resorption depth, with a consequent increase in bone mass.

Studies using bisphosphonates in postmenopausal osteoporosis (Storm et al 1990, Watts et al 1990) have demonstrated an increase in spinal bone mass following treatment with etidronate. Both studies also reported a decrease in the incidence of vertebral fractures after treatment when assessed between 60 and 150 weeks. In contrast patients on the placebo arms of both studies did not have any increase in their bone mineral content, whilst a few even deteriorated. Subsequent studies have shown the beneficial effects of bisphosphonates in osteoporosis due to causes other than postmenopausal osteoporosis.

It is important to remember that bisphosphonates when given in large doses can inhibit normal mineralization and lead to an increase in the incidence of vertebral fractures. There is also a fear of osteomalacia developing. This limits the use of bisphosphonates to about 2 years in clinical practice. However studies using bisphosphonates in cyclical regime have not shown any increase in the incidence of fracture rates. Steiniche et al (1993) have demonstrated the safety of cyclical etidronate over 7 years using histomorphometry in osteoporotic patients. They also showed a clinical correlation of these findings with a decrease in fracture rates. Papapoulos et al (1993) confirm these findings in adolescent osteoporotics on long term uninterrupted treatment with bisphosphonates. These findings are encouraging, as the fears of suppressed mineralization have limited the use of bisphosphonates.

1. 9. 5. Adverse effects of bisphosphonates

Human and animal studies have revealed a very low toxicity profile of bisphosphonates. All bisphosphonates may produce gastrointestinal dysfunction and when given rapidly as intravenous infusions can also cause renal toxicity. Amino derivatives like pamidronate tend to have a higher incidence of side effects as compared to compunds such as etidronate and clodronate. A disturbing side effect is the development of osteomalacia following prolonged high dose use of the bisphosphonates. This is due to an inhibition of normal mineralization. However this is usually not a problem with the regimes generally used in the treatment of osteoporosis. The two studies mentioned above (Steiniche et al 1993, Papapoulos et al 1993) demonstrated the long term safety of bisphosphonates using these treatment schedules. Other adverse effects that have been noted are fairly minor and include effects such as pruritus, erythematous eruptions and transient "acute phase" responses characterised by leucopenia and febrile episodes.

1. 10. AIMS AND OBJECTIVES

The pathophysiology of osteoporosis in ankylosing spondylitis is not well established. Although axial osteoporosis and vertebral deformities have been demonstrated in AS, their relationship to each other is unclear. There is no data regarding the ability of markers of bone formation and resorption to provide information on pathophysiology of osteoporosis and vertebral deformities.

The overall aims of this study were:

1) To establish the presence of osteoporosis in patients with ankylosing spondylitis who have minimal clinical and radiological disease, and, to investigate its effect on vertebral deformities.

2) To investigate whether biochemical markers of bone turnover can provide information on the pathophysiology of osteoporosis and vertebral deformities in mild ankylosing spondylitis, and,

3) To evaluate the effectiveness of bisphosphonate therapy on disease activity, markers of bone turnover and bone mineral density in patients with ankylosing spondylitis who have established osteoporosis.

Chapter 2.

METHODOLOGY

2. 1. Consent

2. 2. Selection of patients

2. 3. Selection of controls

2. 4. Clinical assessments

2. 5. Vertebral deformity definition by morphometry

2. 6. Biochemical markers of bone metabolism

2. 7. Bone mineral density measurements by DXA

2. 8. Statistical analysis

2. 1. CONSENT

All subjects gave informed written consent to take part in the studies described.

All the clinical and laboratory studies were approved by the Bath District Health Authority ethics committee.

2. 2. SELECTION OF PATIENTS

Patients with AS were recruited consecutively from the outpatient clinics of the Royal National Hospital for Rheumatic Diseases, on fulfilling the following selection criteria:

- Patients were required to have primary AS
- They had to be males aged between 20 - 55 years of age,
- All individuals had to have *mild* disease.

Patients were said to have mild disease if:

- Clinically, they had a mobile lumbar spine, with the modified Schober's test ≥ 5 cms, and,
- Radiographically, if their thoracic and lumbar spines were characterised by absent or incipient syndesmophytes, with a radiologic score of ≤ 1 by the criteria of Taylor et al (1991), and, the hip joints were normal.

The diagnosis of AS was based on the modified New York criteria (described in section 1.1.5). In addition, secondary causes of osteoporosis like thyrotoxicosis, hyperparathyroidism, inflammatory bowel disease, multiple myeloma, hypercorticism, Paget's disease and occult malignancies were excluded. No patient was on any medication like steroids, calcium, vitamin D, thiazide diuretics, anticonvulsant medications, fluorides or second line agents like methotrexate, sulphalazine and azathioprine.

2. 3. SELECTION OF CONTROLS

Prior to participation in the study, all subjects gave informed written consent. Secondary causes of osteoporosis like thyrotoxicosis, hyperparathyroidism, inflammatory bowel disease, multiple myeloma, hypercorticism, Paget's disease and occult malignancies were excluded in the subjects who served as controls for BMD and for the biochemical markers of bone turnover.

1. For vertebral morphometry

To compare the risk of vertebral deformities in patients with AS with a group of control subjects without AS, 39 subjects aged 50-60 years were selected from the general population. These individuals had been recruited from primary care registers of local general practitioners and had been participants in a screening survey of vertebral osteoporosis, the European Vertebral Osteoporosis Study (EVOS). Radiographs of the thoracic and lumbar spines were taken for all individuals and fractures of the vertebral bodies were included in the analysis of fracture prevalence.

Age matched control subjects were not used for the case control analyses as it was deemed unsafe to expose young healthy males to the hazards of radiation.

2. For Bone Mineral Density

Z scores are calculated by comparing the BMD of the study subjects with age and sex matched normal control ranges provided by the manufacturers of densitometers (Hologic in this case). The Hologic normal range is an international database. Since, variations in individual population groups are known to occur, ideally two similar population groups should be compared. However, an adequate number of age and sex

matched controls for patients with AS could not be recruited, and therefore it was decided to compare the BMD of the patients with the Hologic normal range.

Before doing so, the differences between the Hologic normal range and the BMD of a local population based control group was evaluated by comparing the two. Therefore, the BMD of 56 healthy male volunteers (members of staff and healthy relatives of patients), aged 20-68 years was compared with the Hologic normal data. No significant difference in the BMD between the two groups was observed. Hence, the Z and T scores of the AS patients were calculated by comparing the BMD (of the AS patients) with the normal range provided by Hologic.

3. For biochemical markers of bone metabolism

Members of staff and healthy relatives of patients were enrolled as control subjects for the biochemical markers of bone metabolism (alkaline phosphatase and its bone isoenzyme, osteocalcin and urinary pyridinium crosslinks). 52 subjects aged 20-55 years agreed to participate in the study. All individuals were required to fill out a questionnaire and all fulfilled the selection criteria which excluded those who had secondary causes of osteoporosis as described above. No individual was on any medication that could affect bone turnover, such as, steroids, vitamin D, fluorides, calcium, thiazide diuretics, and anticonvulsant medications.

Bone sialoprotein was performed in collaboration with the department of Rheumatology, Lund University, Lund, Sweden. The controls for this marker of bone turnover were 25 in number and were selected in the same way as the controls for other biochemical markers.

Hydroxyvitamin D, parathyroid hormone and the sex hormones were assayed as routine and the control ranges of the respective laboratories were applied.

Using separate variance t-tests, no significant difference was found between the mean age of the patients with AS and the control subjects for the biochemical tests.

2. 4. CLINICAL ASSESSMENTS

1. Anthropometric measurements

Heights were measured to the nearest mm and weights were recorded using a clinical balance to the nearest 0.1 kg. For patients who were part of the double blind placebo controlled study, heights and weights were recorded at every visit

2. Diagnosis of AS

The diagnosis of AS in all patients was based on the modified New York criteria (Khan and Linden 1990). All patients had bilateral sacroiliitis ≥ grade 2 along with a history of low back pain for more than 3 months improved by exercise and not relieved by rest. The lumbar flexion in all patients by the modified Schober's criteria (Macrae et al 1969) was ≥ 5 cms.

The radiological scoring of the vertebrae was performed by the criteria of Taylor et al (1991), which is as follows:

0 = Normal

1 = Erosion, Sclerosis or Squaring

2 = Obvious Syndesmophytes

3 = Total bony bridging

Patients with a thoracic and lumbar score of ≤ 1 were included in the study. All patients with a score equal to or higher than 2 were excluded (incipient syndesmophytes were scored 1). All x-rays were read by two individuals independently, of whom one was a radiologist (Dr Gordon Evison) and the other was myself.

3. Evaluation of disease activity

To assess disease activity, a detailed history was recorded in a questionnaire (appendix1) and the patients were subjected to a clinical examination which included metrological assessment. Visual analogue scales for pain in the cervical, thoracic and lumbar spine were recorded, and the duration of early morning stiffness was scored. Peripheral joints were examined for evidence of synovitis and restricted movement.

The assessment was as follows:

Evaluation of early morning stiffness:

The stiffness was scored between 0-5, depending on the duration of the stiffness.

0 = no morning stiffness

1 = stiffness less than 30 minutes

2 = stiffness between 30 minutes - 1 hour

3 = stiffness between 1 - 2 hours

4 = stiffness between 2 - 4 hours

5 = stiffness greater than 4 hours.

Evaluation of pain in the cervical, thoracic and lumbar spine:

Patients were required to mark a 10 cm visual analogue scale (VAS) for an objective assessment of the severity of pain in their spines. Care was taken to blind them to their previous VAS scores to eliminate bias. Patients who were part of the double blind placebo controlled study were assessed with the VAS at every visit.

The peripheral joints were carefully examined by recording a VAS for pain in every joint along with a clinical examination of the joints to establish the presence or absence of synovitis and restricted movement.

Examination of other systems was also performed at each visit to exclude systemic involvement of any kind. Particular attention was paid to the eye to look for evidence of uveitis, and to the genitalia to exclude balanoposthitis.

e) *Metrology*:

Metrological assessment of every patient was performed (during each visit in the double blind placebo controlled study) using standard techniques.

- Cervical spine rotation to the left and right was measured using a custom made goniometer constructed at the Royal National Hospital for Rheumatic Diseases in Bath.
- Lumbar flexion was measured using the modified Schober's method (Macrae et al 1969). In this procedure three marks were made on the skin, with one at the lumbosacral junction, the second 10 cms higher than this and the third 5 cms lower than the lumbosacral mark, i.e. a distance of 15 cms in the erect position. The patient was then asked to bend forwards keeping his knees straight and the distraction between the marks was measured.
- Chest expansion at the level of the nipples was measured using a standard tape measure, following maximum inhalation.
- Tragus to Wall distance was measured with the patient standing such that his heels touched the wall. The distance between the tragus of his ear to the wall was estimated with a sliding scale.
- Intermalleolar distance between the two medial malleoli was measured with a standard tape measure with the lower limbs of the patient abducted maximally.

f) Blood was taken for measurement of C-reactive protein and plasma viscosity by routine methods as an assessment of disease activity.

4. Radiological assessment and monitoring

Standard radiographs of the cervical, thoracic and lumbar spines (antero-posterior and lateral views) and antero-posterior views of the pelvis inclusive of the sacroiliac joints and both hips were recorded. For the patients participating in the double blind placebo controlled study, radiographs were repeated at the end of the 15 month period.

BMD of the lumbar spine and proximal hip was measured in each patient prior to his enrollment in the various studies. For the patients on the drug trial the BMD was

repeated midway through the study, i.e. after 32 weeks, and at the end of the study (65 weeks).

5. Exclusion of secondary causes of osteoporosis

Each patient was questioned in detail about the presence or absence of conditions that could have a bearing on bone metabolism. This included questions about the smoking of tobacco, consumption of alcohol and the duration and frequency of exercising. Conditions like thyrotoxicosis, hypercorticism, hyperparathyroidism, inflammatory bowel diseases, spondyloarthropathy due to Psoriasis and Reiter's syndrome, multiple myeloma, Paget's disease and occult malignancies were excluded. A detailed drug history was elicited and questions were asked specifically about, calcium, fluoride, steroids, methotrexate, sulphasalazine, azathioprine, anti-convulsants, thiazide diuretics and vitamin D.

2. 5. VERTEBRAL FRACTURE DEFINITION BY MORPHOMETRY

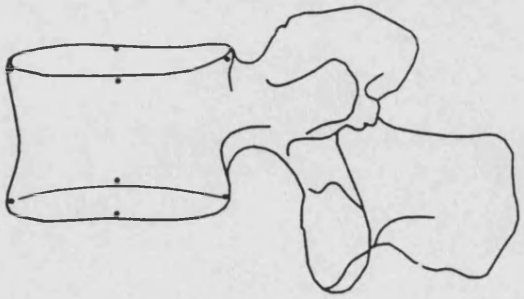
1) Radiographs of the thoracic and lumbar spines

Radiographs of the thoracic and lumbar spine were taken according to a standard protocol. This included details about positioning and radiograph tchniques. The target to film distance was 107 cms. Thoracic films were centered on T7 and the lumbar spine films were centered on L2. Antero-posterior and lateral views of the thoracic and lumbar spines were taken.

2) Morphometry of vertebrae

Each vertebra was marked and measured to ensure consistency of approach. An imaginary line was drawn through the midpoints of the anterior surfaces of the vertebrae. At the junction of this line with the superior and inferior end plates, the anterior points were marked. The posterior points were placed at the base of the posterior angulation of the superior and inferior end plates. Care was taken to make sure the posterior markers lined up. The mid points were placed on the line bisecting the right and left projections of the superior and inferior end plates. Figure 2. 5. 1 shows the point placement for morphometry.

Figure 2. 5. 1. *Illustration of point placement for morphometry.*



The vertebral dimensions of T4 to L5 inclusive, were measured manually with a transparent ruler to the nearest mm. To evaluate the intra-observer reproducibility, 70 vertebrae (5 patients) were measured twice with all previously marked points carefully erased. The difference between each measurement was calculated and a t-test for paired data was performed. No significant difference was observed between the two sets of measurements ($p = 0.63$).

3) Definition of vertebral fracture

Vertebral deformities were defined from T4 - L5 for all patients with AS and the control subjects (section 2.3) according to the morphometric criteria of:

a) Eastell et al (1991), (hereafter referred to as the Eastell method), and,

b) McCloskey et al (1993), (hereafter referred to as the McCloskey method).

a) Fracture definition by the Eastell method

In this method the anterior heights (ha), the posterior heights (hp) and the mid heights (hm) of each vertebra from T4 to L5 are measured from the lateral radiographs. The mid height is the mean of mid height 1 and mid height 2, which are the right and left mid heights.

Based on these measurements three types of deformities are characterised for each vertebra. They are as follows:

Wedge deformity = $(hp - ha)/hp \times 100$

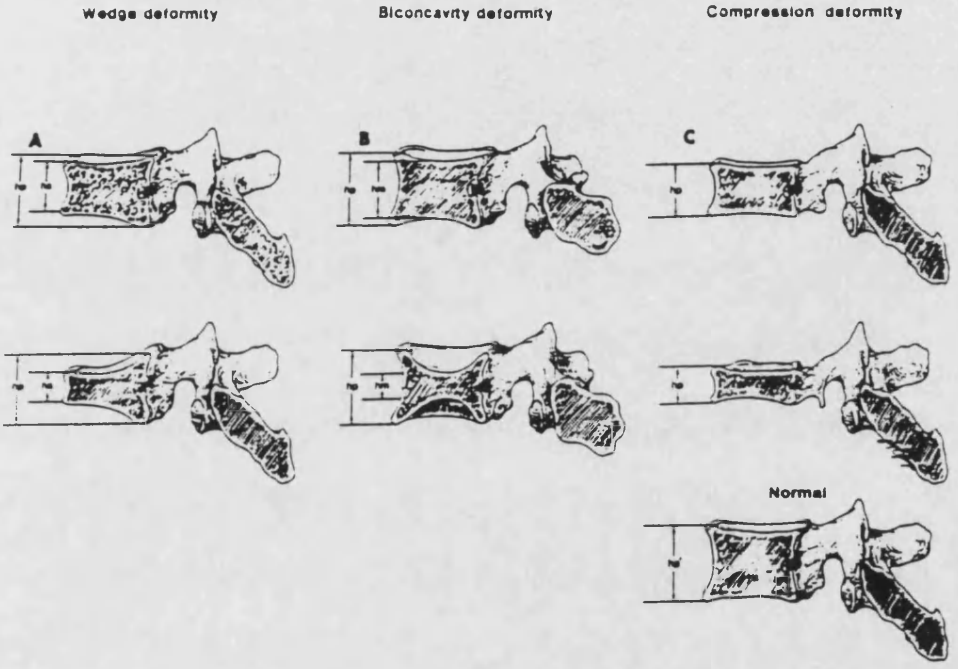
Biconcavity deformity = $(hp - hm)/hp \times 100$

Compression deformity = $(hp' - hp)/hp' \times 100$

where hp' is the posterior height of the cranial or the caudad vertebra in relation to the vertebra concerned.

A vertebra was considered to have grade 1 fracture if it had a deformity that was 3 SD below the mean for that vertebra, and a grade 2 fracture was defined when the deformity was 4 SD below the mean for that vertebra. Figure 2. 5. 2 illustrates the vertebral dimensions and the deformities defined by the Eastell method.

Figure 2. 5. 2. *Illustration of vertebral dimensions and deformities defined by the Eastell method. The upper row shows grade 1 deformities (>3 SD, <4 SD) and the second row illustrates grade 2 deformities (≥4 SD). A normal vertebra is shown in the bottom right.*



b) Fracture definition by the McCloskey method

In this method, at each vertebral level, the mean predicted posterior height (PP) was used to determine the presence or absence of anterior, posterior and central deformities. This method was based on the theory that it was possible to predict the posterior height of one vertebra from the posterior heights of adjacent vertebrae by the use of posterior vertebral height ratios.

The diagnosis of deformities was based on an algorithm that examined each vertebra in turn, from the most cephalic vertebra to the most caudal. Vertebrae with posterior deformities lying above the vertebra under examination were excluded. Lower vertebrae where the posterior heights were reduced in comparison with the vertebra above (>3 SD below the maximum predicted height) were excluded from the calculation of the mean predicted posterior height. The algorithm assumes that four consecutive posterior vertebral deformities would not occur.

In order to minimize the number of false positives, the method defines two criteria which must be fulfilled in order to identify a vertebral deformity.

The classification of vertebra deformities by this method are:
Anterior wedge = A/P and A/PP < (mean A/P - 3 SD)
Central collapse = C/P and C/PP < (mean C/P - 3 SD)
Crush fracture = P/PP < (mean P/PP - 3 SD) and A/PP > (mean A/P - 3 SD)
where PP is the mean predicted posterior height of the vertebra under examination;
A is the anterior height, C is the central height and P is the posterior height.

4) Establishing the prevalence of vertebral fractures

The morphometric method involves measurement of vertebral heights and comparison of the ratios of these heights with individuals without deformity (or in whom deformity has been excluded by a statistical procedure). These normal vertebral height ratios (reference values) need to be vertebral specific, as variation in vertebral shape exists at different vertebral levels.

The prevalence of vertebral deformity was determined in patients with AS and the local controls by deriving reference values from within the population groups (after excluding individuals with deformities), and from a larger UK population based control group. Thus two reference values were used for patients with AS and the control subjects.

Two reference values for each group were used because of differences that exist between populations. The reference values have also been shown to vary between sexes and populations (O'Neill et al 1994). Therefore in clinical epidemiology, it has been suggested that the reference values should ideally be derived from within the individual population groups. However, since the two groups being studied (AS patients and the control subjects) were of small numbers, a second UK population based group (600 men) was used for reference values. It should be pointed out that the UK reference population was measured elsewhere (Berlin) by one of three observers, but, as vertebral height ratios were used for the comparison, this would not affect the case control analyses.

The reference values used were as follows:

- 600 men aged 50-80 years recruited during the course of a population based survey (European Vertebral Osteoporosis Study [EVOS]); used for the patients with AS and the controls.
- Patients with AS; used only for the AS patients.
- Local control subjects; used only for the control group.

In all three cases, the reference values were determined by using a trimming method to exclude deformed vertebrae by the method described by Melton et al (1993). The method entailed dividing the vertebral heights into quartiles and then using an iterative algorithm, removing values greater than 1.5 interquartile ranges above the 75th percentile or below the 25th percentile. This is repeated, with the percentiles and the interquartile ranges being recalculated for the remaining sample till no further

observations qualify for removal. The mean and SD of the trimmed sample are then used as estimates of the mean and SD in unfractured vertebrae.

Using the reference values (normal vertebral height ratios) thus derived and the criteria described for fracture definition by the Eastell and McCloskey methods, the prevalence of vertebral deformities in the AS patients and controls was determined.

2. 6. BIOCHEMICAL MARKERS OF BONE METABOLISM

1) Sample collection and storage

Urine and blood samples from all patients and control subjects were taken between 8:30 am and 10:00 am following an overnight fast.

Blood samples were collected from a cubital vein into relevant vacutainers, labelled, and allowed to clot for 30 minutes prior to centrifugation at 2000 rpm for 15 minutes. Serum was placed in 5 ml plastic aliquots and stored at -70°C prior to analysis. All samples were processed and stored within 1 hour of collection. Before the samples were used to study laboratory markers of bone turnover they were examined for evidence of hemolysis.

The collection of urine samples depended on the study that was being requested. For 24 hour calcium and cortisol measurements all samples were timed 24 hour collections. For calcium creatinine ratios and collagen pyridinium cross links, 2 hour second void samples were collected following an overnight fast. All samples were stored in 5ml sterile plastic aliquots after determining the volume voided (for 24 hour studies).

2) Pyridinium crosslinks of collagen in urine

Materials

Acetonitrile and water were HPLC grade (Merck Chemicals Ltd., Poole, Dorset). The hepatofluorobutyric acid (HFBA) was sequenal grade (Pierce and Warriner, UK, Ltd.).The HPLC column used was 33 × 4.6 mm (LDC, Staffordshire, UK), and the columns were packed with 3 μm octadecyldimethysilane (ODS) (Phasesep Ltd., Clwyd, Wales). Analytical grade n-butanol, acetic acid and hydrochloric acid were used. CFI cellulose (Whatman Ltd., Maidstone, Kent, UK) columns were prepared using disposable polyprep columns (Biorad Labs Inc. Hemel Hempstead, UK). A centrifugal evaporator (Jouan Ltd., Tring, Herts, UK) was used to lyophilise the samples.

Methods

The urinary pyridinium crosslinks were determined by a modification of the method described by Black et al (1988). The details are given below.

Hydrolysis

Duplicate urine samples (250 μL) were placed in 100 × 13 mm screw capped bottles and an equal volume of 6 M Hydrochloric (HCL) was added for hydrolysis. The tubes were vortexed and the samples hydrolysed for 18 hours at 110°C.

Preparation of standard

a) Preparation of pyridinoline crosslinks from bovine bone

The standard was prepared from bovine bone as described by [Black et al (1988) (Dr. D. Black Glaxo Ltd., Greenford UK)]. 140 grams of bovine articular cartilage was stirred for a week in 1.5l 0.5 M EDTA, and then freeze dried. The cartilage was then refluxed in 1500 ml of 6 M HCL for 16 hours at 116°C. The volume was reduced by distillation to 500 ml. Equal volumes of glacial acetic acid, 5% cellulose slurry in 4:1:1 solvent, and four volumes of n-butanol were added to the hydrolysate in a 2L container. The mixture was stirred for 30 minutes at room temperature, filtered and then washed with 5l of butanolic eluent. Pyridinoline was then eluted with 150 ml

distilled water and the aqueous fraction was lyophilised and resuspended in 10 ml of 0.05 M HCL. For further purification, 5gms of sodium cellulose phosphate powder in 20 ml distilled water was added to a 20 ml syringe barrel stoppered with a glass wool to a depth of 5 cms. The column was then washed with 10 ml 1mM HCL, and 1 ml of the sample was added at pH > 4. The column was finally washed with increasing concentrations of HCL and the pyridinoline crosslinks were eluted with 10 ml of 6 M HCL.

Cellulose partition chromatography

500 µL of glacial acetic acid, 500 µL of 5% CFI slurry in n-butanol : acetic acid : water (4 : 1 : 1) and 2ml n-butanol were added to each hydrolysed sample. The prepared hydrolysates were then thoroughly mixed and poured onto CF1 columns prepared from 5 ml of 5% CF1 slurry in disposable polyprep columns. The hydrolysis tubes were then washed with 2 ml of the butanolic eluent (4:1:1 solvent mixture) and added to the columns which were then washed with two 10 ml aliquots of the butanolic eluent. The crosslinks were then eluted with 5 ml of distilled water into 5 ml tubes. The butanolic phase (top layer) was aspirated and the aqueous phase was evaporated to dryness. Each sample was then reconstituted in 250 µL of 1% HFBA and then added to the HPLC column.

High Performance Liquid Chromatography (HPLC)

The HPLC apparatus consists of two Jasco-PU pumps (Ciba-Corning, Halstead, Essex, UK). The 2mm glass lined column was packed with inertsil (Scientific Glass Engineering Ltd., UK). It was equipped with a high pressure mixer, a Waters 712 autosampler (Millipore-Waters, Milford, MA, USA), a Jasco 821-FP fluorescence detector and a Waters 820 data collection system. Before use, isocratic separation was achieved within 4 mins using two solvents, A (88%) and B (12%). Solvent A was 0.01 M HFBA, pH 2.70 (2.64 ml HFBA in 1L distilled water), and, Solvent B was 75% acetonitrile in distilled water. Running the solvents helped to achieve a stable baseline. 20 µL of the sample was added onto the columns which were washed every 10 mins with solvent B at 1 ml/min for 3 mins, and recalibrated to maintain column efficiency.

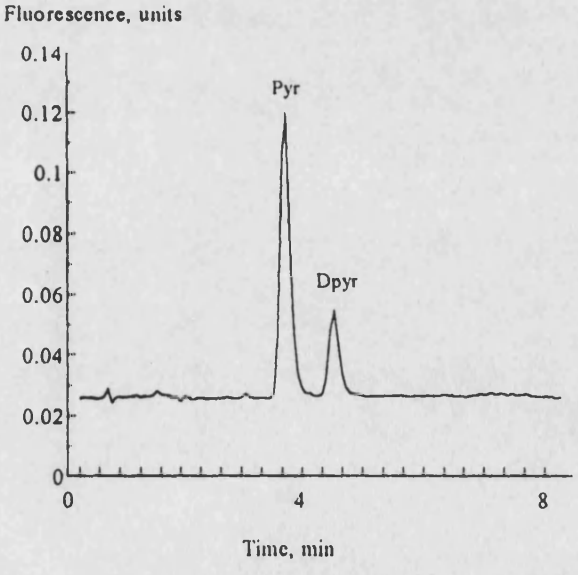
The fluorescence was monitored with the excitation wavelength at 295 nm, and emission wavelength at 395 nm. Quantitation was achieved using external standardisation. Samples were run in duplicates and were reanalysed if the difference between the duplicates was more than 10%.

Expression of results

Pyridinoline and deoxypyridinoline excretion in the fasting two hour samples were expressed as ratios to urinary creatinine. Urinary creatinine was performed by the Jaffe method.

An example of a chromatogram of the crosslink standard is shown in figure 2.6.1. demonstrating the separation achieved with this method.

Figure 2. 6. 1. *Chromatogram of an external crosslink standard illustrating the timing of elution of the crosslinks and the separation achievd using the method described.*



3) Osteocalcin measurement by ELISA

Materials

Microwell plates (DAKO, Ltd., UK) of which six are white and six are optically clear were provided for premixing of the standards, control and samples. Bovine osteocalcin standards (DAKO, Ltd., UK) were also provided with peroxidase-conjugated streptavidin, and a highly purified bovine osteocalcin labelled with biotin (biotinylated osteocalcin). Washing buffer, (1.25 M tris/HCl, 2.5 M NaCl, 5% Tween 20, pH 7.2 preserved with 0.01% Bronidox. Two substrate solutions, tetramethylbenzidine (TMB) and hydrogen peroxide were included with the kit. Sulphuric acid 2M, was used as a stopping solution.

Principle of the assay

The DAKO osteocalcin ELISA is a competitive assay where samples and biotinylated osteocalcin were incubated simultaneously in antibody coated microwells.

Osteocalcin standards, a control curve and patient specimens were premixed with biotinylated osteocalcin in white microwells. The sample mixtures were then transferred to optically-clear microwells precoated with antiosteocalcin. During a 1 hour incubation period osteocalcin and biotinylated osteocalcin competetively bound to the limited number of solid phase antibodies. Following a washing step, where unbound material was removed, peroxidase-conjugated streptavidin was added to the optically clear microwells. A 15 minute incubation period followed this during which the streptavidin bound strongly to biotinylated osteocalcin already captured by the solid-phase antibody. Following a second washing step, where unreacted streptavidin was removed, a chromogenic substrate was added to the wells. The more osteocalcin the sample contained, the less colour was developed. The colour development was stopped by the addition of sulphuric acid and the absorbance at 450 nm was measured. A standard curve was prepared from the 6 osteocalcin standards and the concentration of osteocalcin in the patient samples was determined by interpolation of values on a semi-logarithmic graph paper against this curve.

Method

The wells in the white strips were used to premix 200 µL of the biotinylated osteocalcin with 50 µL of the standard provided, the bovine osteocalcin (used to determine the curve control), and the serum of patients. The mixture was thoroughly mixed and 200 µL of the mixed contents were transferred to the corresponding wells on the optically clear wells in the clear strips. The mixture was then incubated for 1 hour on a microwell plate mixing apparatus. Following this the clear strips were washed well and the mixture was then incubated for 15 minutes with 200 µL of peroxidase-conjugated streptavidin. The wells were washed again and incubated in the dark with 200 µL of the chromogenic substrate solution. The activity was stopped after 30 minutes with 2 M sulphuric acid and the absorbance was read on a Perkin Elmer Fluorescence Spectrophotometer LS 5B equipped with a plate reader (Perkin Elmer Ltd., Beaconsfield, UK) within one hour at 450 nm or at 450 nm with 650 nm as reference wavelength.

Calculation of results

The standard curve was plotted using absorbance as an ordinate. This was done by calculating the corrected mean absorbance and plotting these corrected mean absorbances on a semi logarithmic graph paper against the corresponding osteocalcin concentrations. The osteocalcin concentrations of the curve control and of each patient was then calculated by extrapolation on this curve.

All samples were run in duplicates and the test was repeated if the values differed by more than 10%.

4) Bone Sialoprotein (BSP) by ELISA

Purification of antigen

Human BSP was prepared from powdered bone of femoral heads of patients undergoing hip joint replacement as described by Franzen and Heinegard (1985).

Preparation of antibodies

Antibodies directed against human BSP were prepared in a rabbit. Initial immunization was carried out with 250 μg of BSP in Freund's complete adjuvant (Difco Labs., Detroit, MI, USA). After a month, one booster dose of 250 μg BSP in Freund's incomplete adjuvant (Difco Labs., Detroit, MI, USA) was given, whereafter the antibody titre was sufficient. The antiserum was used without further purification.

ELISA for BSP

An ELISA with specificity for BSP was developed. The white plates were Dynatech Microfluor M119W (Dynatech Labs. Inc., Alexandria, Virginia, USA). All procedures were performed at room temperature. The wells were coated for 24 hours with 200 μL of BSP, dilution 0.1 μg/ml in 20 mM Tris, 50 mM $CaCl_2$, pH 7.5. The plates were then rinsed with 0.15 M NaCl and 0.05% (w/v) Tween 20 to remove unbound BSP. The plates were then after-coated with 2mg/ml bovine serum albumin (Serva AG, Heidelberg, Germany) in 20 mM Tris, 50 mM $Cacl_2$, pH 7.5, for 1 hour to reduce nonspecific binding, and were then again rinsed as above.

110 μL of the serum samples of the patients was then mixed with an equal volume of a 1/800 dilution of rabbit anti-human BSP in 0.1 M NaCl, 5 mM CaCl2, 50 mM Tris, 0.05% Tween 20, pH 7.5. After preincubation for 24 hours, 200 μL of the mixture was the added to the coated wells of the microtitre plate. The plates were then incubated for 1 hour and then rinsed. Pig anti-rabbit-IgG in a 200 μL dilution with alkaline phosphatase (Orion Diagnostica, Helsinki, Finland) in 0.1 M NaCl, 5 mM CaCl2, 50 mM Tris, 0.05% Tween 20 and 2 mg/ml bovine serum albumin (pH 7.5) was added. The plates were further incubated for 1 hour and then rinsed. Following

this, 200 μL of the enzyme substrate, 2 μM methyl-umbellipherylphosphate (Sigma, St. Louis, MO, USA) in 0.05 M 2-amino-2-methyl-1,3-propandiol, 0.05 mM MgCl2, pH 9.6 was added.

The fluorescence was measured in a Perkin Elmer Fluorescence Spectrophotometer LS 5B equipped with a plate reader (Perkin Elmer Ltd., Beaconsfield, UK) immediately after the substrate addition and 1 hour after incubation. A standard curve using dilutions of human BSP was included in each microtitre plate. All samples were analyzed in triplicate and the mean value was used.

5) Alkaline phosphatase

Alkaline phosphatase was measured by using 2-amino-2-methyl-1-propranolol (AMP) buffer on a Technicon RA-XT. (Technicon Instruments Corpn. New York, USA).

Principles of the procedure

The reaction was initiated by the addition of the patients serum to the reagent. The AMP buffer increased enzyme activity through transphosphorylation. The hydroxyl group on the buffer attracted the phosphate product of the reaction, thereby increasing the rate of reaction. The sample was then added to a p-nitrophenyl phosphate (PNPP) substrate. The AMP buffer maintains the reaction pH at 10.3-10.4. Alkaline phosphatase hydrolyses the PNPP substrate to form a highly coloured p-nitrophenol, which is then measured.

Method

50 μL of the sample was added to 1500 μL of the reagent. It was vortexed and incubated at 37°C. The absorbance was read at 405 nm at 30 seconds and then at 1 minute intervals for 4 minutes. The mean absorbance difference per minute was then calculated (Δabs/min). The test was run in duplicates and was repeated if the values differed from each other by more than 10%.

Calculation of results

The alkaline phosphatase activity was measured by 1667 × (Δabs/min).

6) Bone specific isoenzyme of alkaline phosphatase

The bone specific isoenzyme of alkaline phosphatase was measured by using a modification of the method described by Rosalki and Foo (1984), which used wheat germ lectin to precipitate the bone specific isoenzyme.

Method

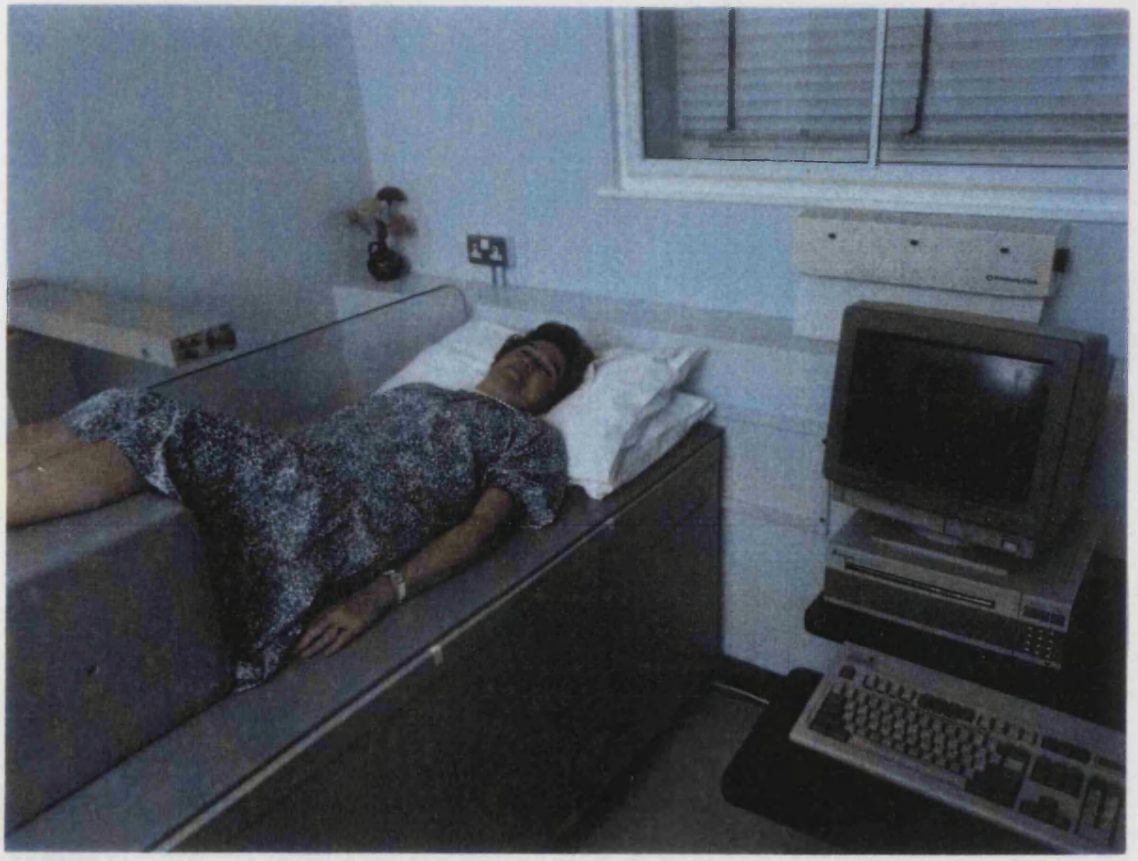
The total activity of alkaline phosphatase was first measured by the method described above and then bone alkaline phosphatase was determined by using a precipitant - wheat germ lectin, to precipitate the bone specific isoenzyme. The precipitation of bone alkaline phosphatase was carried out as follows: equal volumes (0.1ml) of the serum and the lectin solutions were mixed and centrifuged for 2000 g for 15 mins. The activity of alkaline phosphatse in the remaining supernatant was then determined. The bone alkaline phosphatase activity was then calculated by subtracting the alkaline phosphatase activity of the supernatant from the total alkaline phosphatase measured earlier. Quality control was achieved by using a special control serum based on human serum whose values were predetermined. The samples were run in duplicates and the test was repeated if the values differed by more than 10%.

2. 7. BONE MINERAL DENSITY MEASUREMENTS BY DXA

Methods

For the study, measurements of the bone mineral density (BMD) of the lumbar spine and femoral neck were carried out using a Hologic QDR 1000 (Hologic Inc., Waltham MA) (fig. 2.7.1.).

Figure 2. 7. 1. *The Hologic QDR 1000 dual energy x-ray absorptiometer. Illustration of position for a lumbar spine scan.*



The scans were performed according to the instructions given by the manufacturers. Patient positioning for BMD measurements of the lumbar spine involved laying the patient in a supine position and placing a cushion with sufficient height to eliminate lumbar lordosis of the spine (fig 2.7.1). For measuring the BMD of the femoral neck, the leg was abducted by 10-15°, and internally rotated by approximately 15°. A positioning device is usually used to achieve the correct positioning of the leg.

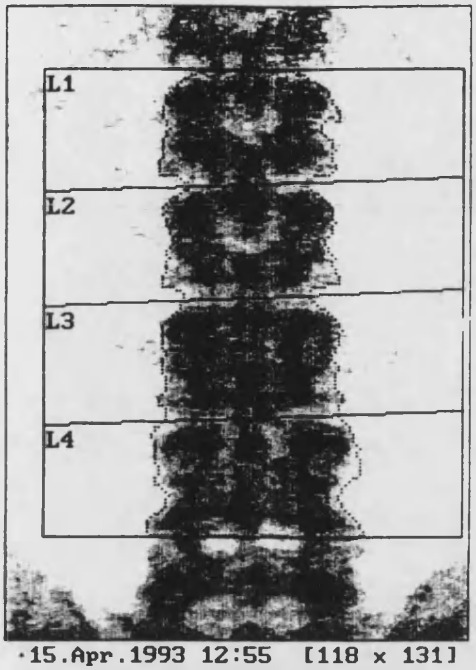
Results of the scans performed are usually expressed as the bone mineral content (BMC, grams) and bone mineral density (BMD, gms/cm^2) (fig.2.7.2). Figure 2.7.2.a. shows results of a lumbar spine scan and figure 2.7.2.b. shows results of a scan of the proximal hip.

For the lumbar spine the scan included L1-L4 (fig 2.7.2.a) and for the hip, the neck of femur, trochanteric area, intertrochanteric area, Wards triangle and the total area (fig 2.7.2.b). The global BMD was expressed as a graph and compared to the manufacturer's reference range. In the graph, the reference range of the lower limit of young adult normals is plotted as a dotted line. The values for each region scanned are shown in a table as a percentage of the value expected for age (Z score), and as a comparison with young normals (T score). These values were expressed as standard deviations (SD) from the mean.

Figure 2. 7. 2. a. *DXA scan of the lumbar spine by Hologic QDR 1000.*

CLINICAL MEASUREMENT - RNHRD BATH

k = 1.227 d0 = 108.9(1.000H)



·15.Apr.1993 12:55 [118 x 131]
Hologic QDR 1000 (S/N 250)
Lumbar Spine V4.47

A0415930C Thu 15.Apr.1993 12:10
Name:
Comment: AS ETIDRONATE STUDY
I.D.: Sex: M
S.S.#: - - Ethnic: W
ZIPCode: Height: 164.50 cm
Scan Code: LB Weight: 67.70 kg
BirthDate: 02.May.54 Age: 38
Physician:

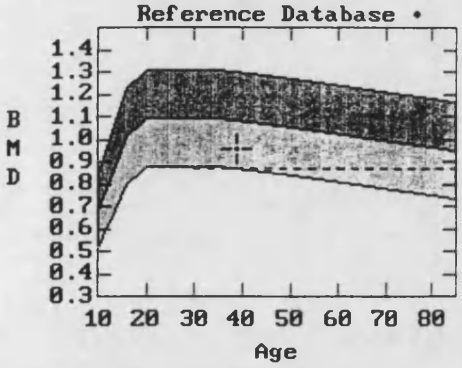
TOTAL BMD CV FOR L1 - L4 1.0%

C.F. 1.005 1.033 1.000

Region	Area (cm^2)	BMC (grams)	BMD (gms/cm^2)
L1	14.07	11.97	0.851
L2	13.76	12.68	0.922
L3	15.64	15.62	0.999
L4	17.87	18.32	1.026
TOTAL	61.34	58.60	0.955

HOLOGIC

Lumbar Spine
Reference Database •



BMD(L1-L4) = 0.955 g/cm^2

A0415930C Thu 15.Apr.1993 12:10
Name:
Comment: AS ETIDRONATE STUDY
I.D.: Sex: M
S.S.#: - - Ethnic: W
ZIPCode: Height: 164.50 cm
Scan Code: LB Weight: 67.70 kg
BirthDate: 02.May.54 Age: 38
Physician:

Region	BMD	T(30.0)		Z	
L1	0.851	-1.43	84%	-1.43	84%
L2	0.922	-1.57	84%	-1.57	84%
L3	0.999	-0.94	91%	-0.94	91%
L4	1.026	-1.09	90%	-1.09	90%
L1-L4	0.955	-1.23	88%	-1.15	88%

◆ Age and sex matched
T = peak bone mass
Z = age matched TK 04 Nov 91

HOLOGIC

Figure 2. 7. 2. b. *DXA scan of the proximal hip by Hologic QDR 1000.*

CLINICAL MEASUREMENT - RNHRD BATH

k = 1.244 d0 = 118.1(1.000H)

·06.Nov 13:04 [109 x 121]
Holog 1000 (S/N 250)
Hip V4.47

A1106920B Fri 06.Nov.1992 12:24
Name:
Comment: AS ETIDRONATE STUDY
I.D.: Sex: M
S.S.#: - - Ethnic: W
ZIPCode: Height: 167.80 cm
Scan Code: LB Weight: 52.70 kg
BirthDate: 18.Jun.65 Age: 27
Physician:

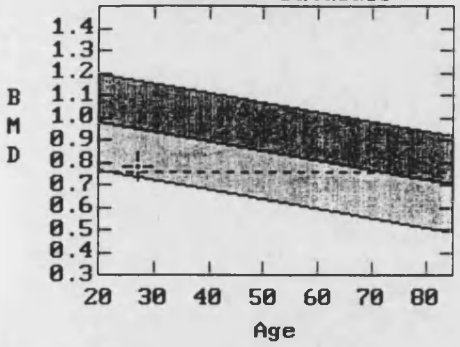
C.F. 1.005 1.031 1.000

Region	Area (cm^2)	BMC (grams)	BMD (gms/cm^2)
Neck	5.39	4.18	0.775
Troch	9.42	5.65	0.600
Inter	25.88	22.62	0.874
TOTAL	40.69	32.46	0.798
Ward's	1.08	0.67	0.619

Midline (104,138)-(22, 72)
Neck 51 x 16 at [-29, 16]
Troch -16 x 42 at [0, 0]
Ward's 11 x 11 at [-6, 2]

HOLOGIC

Right Hip
Reference Database •



BMD(Neck[R]) = 0.775 g/cm^2

Region	BMD	T		Z	
Neck	0.775	-1.85 (20.0)	79%	-1.56	82%
Troch	0.600	-1.79 (20.0)	75%	-1.67	77%
Inter	0.874	-2.46 (20.0)	70%	-2.31	72%
TOTAL	0.798	-2.11 (20.0)	74%	-1.95	76%
Ward's	0.619	-1.77 (20.0)	74%	-1.39	79%

• Age and sex matched
T = peak bone mass
Z = age matched TK 25 Oct 91

A1106920B Fri 06.Nov.1992 12:24
Name:
Comment: AS ETIDRONATE STUDY
I.D.: Sex: M
S.S.#: - - Ethnic: W
ZIPCode: Height: 167.80 cm
Scan Code: LB Weight: 52.70 kg
BirthDate: 18.Jun.65 Age: 27
Physician:

HOLOGIC

For BMD measurements of the lumbar spine, care was taken to exclude lumbar vertebrae from analysis that were architecturally deformed.

Although bone densitometry was performed on all possible sites around the hip, BMD measurements of the femoral neck were considered for the study in preference to other areas around the hip. This was due to the fact that precision of this site was greater than that of Ward's triangle or the trochanteric sites. Cummings et al (1993) have shown that femoral neck BMD has similar diagnostic accuracy in the prediction of hip fractures as compared to other sites of BMD measurement.

Reproducibility of BMD measurements by DXA

Twenty subjects were measured twice, with repositioning between each scan and the reproducibility was calculated. The precision error was shown as a percentage. The reproducibility was calculated as the ratio of standard deviation of the duplicate measurements to the mean BMD, expressed as a percentage. The results of the precision studies are as follows:

Table 2. 7. 1.. *Reproducibility of DXA measurements. Precision error in percentage (%).*

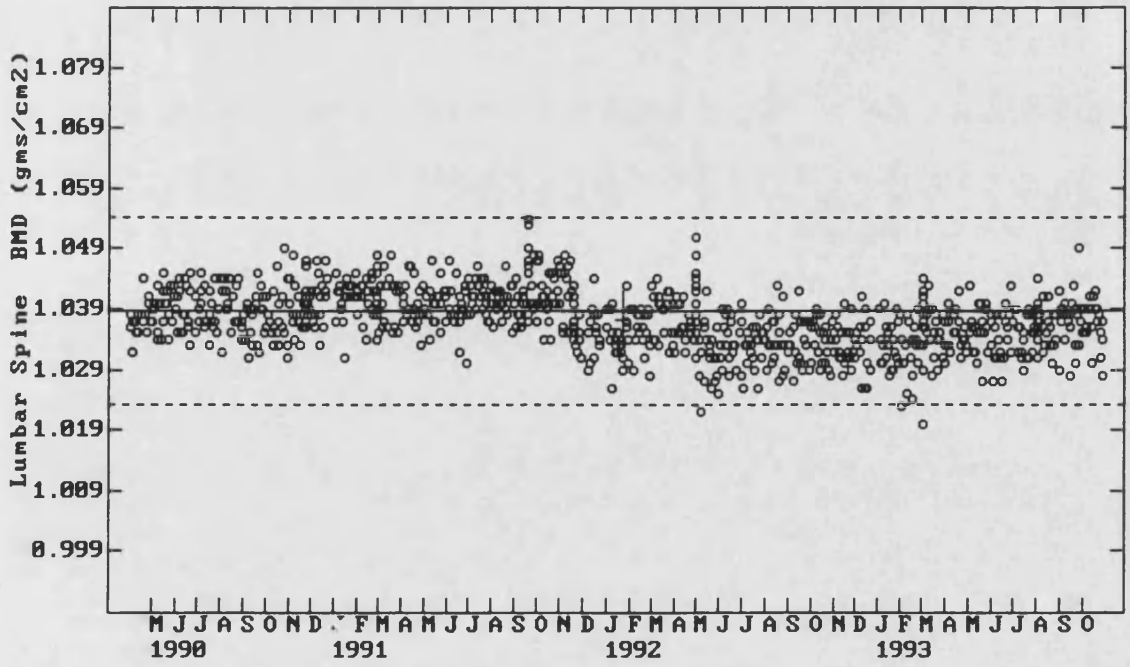
Measurement Site	*Precision Error (%)*
Lumbar spine (L1-L4)	1.40
Femoral Neck	2.90

Quality control of bone mineral density measurements

Each QDR 1000 is supplied with an anthropomorphic spine phantom that serves as a test object. Daily scans of this phantom were made and the measured values of BMC, projected area and BMD (BMC/Area) are recorded on a database. Inspection for any trends in these daily measurements enables the consistency of calibrations to be checked. At the time of manufacture, each system is calibrated against a master phantom that contains sample of bone equivalent medium with BMD values of approximately 0.6, 1.0 and 1.6 gm/cm^2. After calibration each QDR 1000 was assigned an anthropomorphic spine phantom. Any subsequent variation in calibration can be detected by reference to this site phantom.

As recommended, *in vitro* precision was monitored for the Hologic QDR 1000 each day by scanning the manufacturer's phantom. Figure 2.7.3 shows the results of the phantom measurement over a four year period, from 1990 to 1993.

Figure 2. 7. 3. *Measurements of the spine phantom on the Hologic QDR 1000 densitometer over 4 years.*



Reference Values	Plot Statistics
all spine phantoms	n = 952
mean = 1.0387 gms/cm2	mean = 1.0372 gms/cm2
S.D. = 0.0033 gms/cm2	S.D. = 0.0048 gms/cm2
limits = ±1.5% of mean	cv = 0.46%

(all)

HOLOGIC

Diagnosis of osteoporosis

Subjects included in this study were considered to have osteoporosis when the Z score using age and sex matched controls was greater than 1 standard deviation below the mean for that individual.

Recent guidelines laid down by the WHO suggest considerably stringent criteria for the diagnosis of osteoporosis in *adult women.* Recommendations made by Kanis et al (1994) require the value of bone mineral density to be greater than 2.5 SD below the young adult mean value to diagnose osteoporosis. They do not recommend these criteria for the diagnosis of osteoporosis in men. There is insufficient data to establish criteria for the diagnosis of osteoporosis in men presently, as per WHO recommendations (Kanis et al 1994).

Since this study had already got underway well before these guidelines were published, the criteria used to diagnose osteoporosis for the patients with AS (all males) was maintained at Z scores more than 1 SD below the mean for age matched controls.

2. 8. STATISTICAL ANALYSIS

Statistical analyses were performed using SPSS for Windows.

Logarithmic transformation of variables was used to approximate a normal distribution when necessary and non-parametric statistical analyses were used whenever the variables were not normally distributed. The details of the various analyses are given in the relevant sections

5% was taken as the level of significance and the significance levels were denoted as $p<0.05$, $p<0.01$ and $p<0.001$.

CHAPTER 3 - THE PREVALENCE OF VERTEBRAL DEFORMITIES IN MILD ANKYLOSING SPONDYLITIS AND ITS RELATIONSHIP TO BONE MINERAL DENSITY

Summary

Background and aims

Vertebral fractures are known complications of ankylosing spondylitis (AS), however, because a large proportion of fractures are asymptomatic these may often go unrecognized. The aims of this study were to determine the BMD of the lumbar spine and femoral neck in patients with mild AS, to establish the prevalence of vertebral deformities in mild AS and compare this with a population based control group, and, to determine the relationship between BMD and vertebral deformities.

Methods

All subjects had spinal radiographs and BMD of the lumbar spine and femoral neck. Lateral spine radiographs were evaluated morphometrically and the presence of vertebral deformity was defined using the Eastell and McCloskey methods.

Results

BMD of the lumbar spine and femoral neck in AS was more than 1 SD below the age matched mean in 62.1% and 48.5% of patients respectively. The prevalence of deformities defined by the McCloskey method was 16.7% and by the Eastell method was 24.2% (grade 1) and 7.6% (grade 2). Patients with deformities were older (mean age 41.4 *Vs* 37.8 years, $p = 0.17$) and had longer disease duration (12.4 *Vs* 9.3 years, $p<0.05$) than those without deformities. Compared to the control population, patients with AS had an increased risk for deformity; odds ratio for the McCloskey method, 5.92 (95% CI 1.4-23.8) and for the Eastell method 6.5 (95% CI 1.0-41.2). There was no relationship between BMD of the lumbar spine and femoral neck with vertebral deformities.

Conclusions

Axial osteoporosis and vertebral deformities are a feature of mild AS and the risk of deformities in patients with AS increases with age and disease duration. The lack of relationship between BMD and vertebral deformities in AS suggests that other factors in addition to low BMD may be implicated in the pathogenesis of the deformities.

Introduction

Vertebral osteoporosis and fractures are features of advanced AS and have been related to spinal immobility. Though the occurrence of osteoporosis is considered common, there is considerable disagreement about the prevalence of fractures, with some authors observing a low prevalence (Wilkinson et al 1958, Hunter et al 1978; 1983, Hanson et al 1971) and others describing a higher prevalence with excess risk (Dilsen 1964, Ralston et al 1990, Cooper et al 1994, Donnelly et al 1994). The only study to date to address the occurrence of deformities in AS using recommended morphometric criteria (Donnelly et al 1994) found a higher prevalence in patients with AS. However the reference values were derived from a different sex population. There is no data on the prevalence of vertebral fractures in AS with reference values derived from a population of the same sex or using a sex matched comparison group.

The presence of osteoporosis in patients with mild AS has been observed by numerous researchers with the use of dual photon absorptiometers and DXA (Will et al 1989, Devogelaer et al 1992, Mitra et al 1994, Mullaji et al 1994). BMD is a good determinant of vertebral fractures in women with postmenopausal osteoporosis (Cummings et al 1993, Wasnich et al 1985). However, the only study to look at the relationship between BMD and vertebral fractures in AS found none (Donnelly et al 1994). It must be said though that the latter study consisted of a heterogenous patient population of which almost half had advanced spinal changes, and no data on drugs was given. No study has looked at the relationship between BMD and vertebral fractures in patients with *mild* AS as yet.

The *aims* of the study were to:

1. To determine the BMD of the lumbar spine and femoral neck in mild AS,
2. To establish the prevalence of vertebral deformities in mild AS and compare this with a population based control group,
3. To determine the relationship between BMD and vertebral deformities in mild AS.

Methods

Subjects

66 patients with AS and 39 controls were recruited for this study as described in sections 2.2 and 2.3 respectively. All patients had detailed clinical assessment as described in section 2.4. Radiographs of the spine (antero-posterior and lateral views) and BMD of the lumbar spine and femoral neck were performed in all subjects within three months of each other.

Vertebral deformity definition - criteria used

Vertebral deformities were defined by morphometric techniques using two methods, Eastell and McCloskey (described in section 2.5). Two reference values derived from different sources were used to define vertebral deformity for the AS patients and the control group (described in section 2.5) and were as follows:

For the patients with AS:

- UK reference population (hereafter referred to as UKR)
- The AS group itself (hereafter referred to as ASR)

For the control group:

- UK reference population (UKR)
- The control population itself (hereafter referred to as CR)

Note: For all subsequent analyses other than the prevalence of deformities, the UK reference values (UKR) were used.

Analyses

Bone mineral density

BMD Z and T scores were calculated by comparing the values from AS patients with normal ranges provided by the manufacturers, Hologic. The diagnosis of osteoporosis was based on the BMD being more than 1 SD below the normal mean for age matched controls. One tailed t tests were performed to compare the BMD of AS patients with the Hologic normal range.

Vertebral deformity

The prevalence of vertebral deformities in AS was determined using both methods and two reference values for each method. To explore the distribution and type of deformities, the McCloskey method was used along with the UKR. The prevalence of deformities in AS was compared with that of the control group for each method using the UKR to define deformities. The risk of deformities in patients with AS as compared to the control group was expressed as odds ratios and 95% confidence intervals.

Relationship between BMD and vertebral deformities in AS

To determine the relationship between BMD and vertebral deformities in AS, the latter were defined by the Eastell and McCloskey methods using UKR. The BMD of the patients with deformities was compared with those without.

Relationship between BMD and deformities with age and disease duration

Spearman's correlation coefficients were used to determine the relationship between BMD, age and disease duration. Mann Whitney tests were used to determine the relationship between deformities and these parameters. Significance was defined by $p < 0.05$.

Statistics

The statistical tests used for this study were Mann Whitney tests, Spearman's correlation coefficients and one tailed t tests. P values less than 0.05 were considered to be significant.

Results

Descriptive characteristics of AS patients (Table 3.1)

The demographic and radiographic characteristics of the 66 patients with AS are shown in table 3.1. The median age was 37.75 years (range 20-52 years) and median disease duration was 9.85 years (1-19). Radiologically they had no syndesmophytes in their lumbar and thoracic spines with the median radiologic scores being 1.00 (0-1) and 1.00 (0-1) respectively. All patients had mobile lumbar spines.

Table 3. 1. *Demographic, clinical and radiographic features of patients with AS.*

Variable	Median	Range
Demographic features		
Age (years)	37.75	(20-52)
Duration (years)	9.85	(1-22)
Body mass index [weight(kg)/height(m^2)]	24.98	(18.72-32.91)
Clinical features		
VAS-Cervical spine (cm)	3.00	(0-8.5)
VAS-Thoracic spine (cm)	1.84	(0-8)
VAS-Lumbar spine (cm)	3.80	(0-8.7)
Early morning stiffness (mins)	60 mins	0-120 mins
Tragus-Wall (cms)	9.85	(7-22.5)
Chest Expansion (cms)	4.75	(1-8.5)
Modified Schober's (cms)	7.00	(3-10)
Intermalleolar distance (cms)	115.00	(76-148)
Radiographic scores		
XRay-Sacroiliac Joint-Right	3.00	(1-4)
XRay-Sacroiliac Joint-Left	3.00	(1-4)
XRay-Lumbar spine	1.00	(0-1)
XRay-Thoracic spine	1.00	(0-1)
XRay-Cervical spine	1.50	(0-3)
XRay-Right hip	0.00	(0-1)
XRay-Left hip	0.00	(0-1)

VAS - visual analogue scale

BMD in AS patients (Table 3.2)

Patients with AS had significantly lower lumbar spine [mean Z score, -1 (95% CI -0.5, +0.16)] and femoral neck [mean Z score, -0.7 (95% CI -0.41, +0.2)] Z scores compared with age matched controls ($p < 0.001$) (table 3.2).

Table 3. 2. *Mean BMD of the lumbar spine and femoral neck*

Variable	Mean (SD)	95% Confidence Interval
BMD		
Lumbar spine (gm/cm^2)	0.97 (0.14)	-0.02, +0.06
Femoral neck (gm/cm^2)	0.82 (0.13)	-0.02, +0.06
Z score		
Lumbar spine (L1-4)	-1.00 (1.30)	-0.50, +0.16
Femoral neck	-0.70 (1.20)	-0.41, +0.20
T score		
Lumbar spine (L1-4)	-1.10 (1.30)	-0.50, +0.14
Femoral neck	-1.40 (1.20)	-0.51, +0.09

BMD of the lumbar spine and femoral neck was more than 1 SD below the age matched mean in 62.1% and 48.5% of patients respectively. The spread of T and Z scores are shown in figures 3.1 and 3.2 respectively, the majority of the values lying below the mean at 0.

Figure 3. 1. *T scores of the lumbar spine and femoral neck in patients with AS*

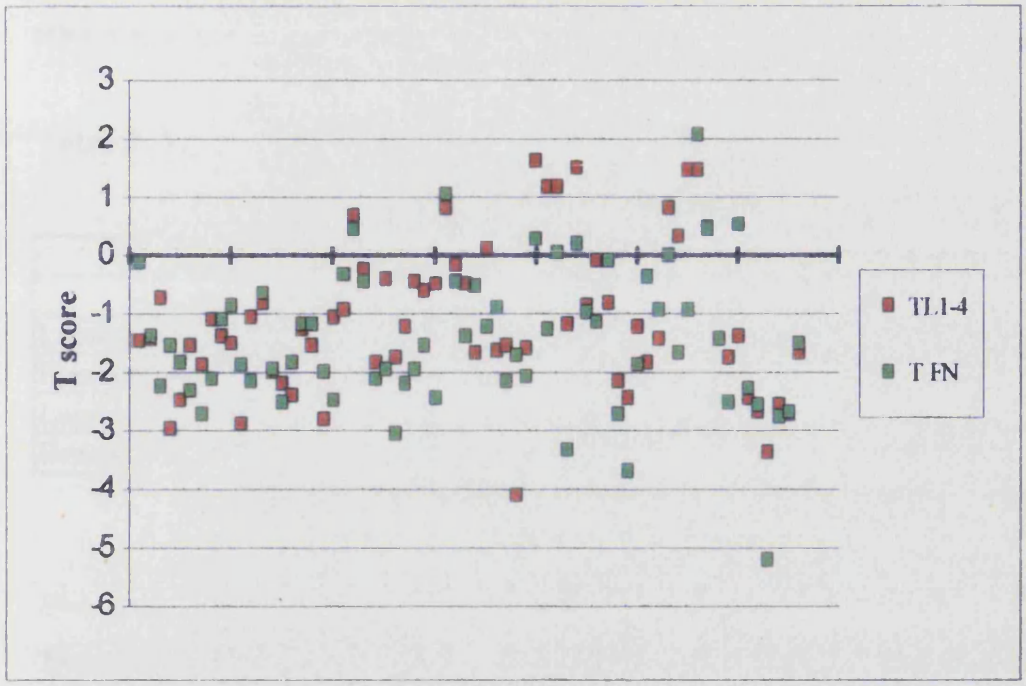
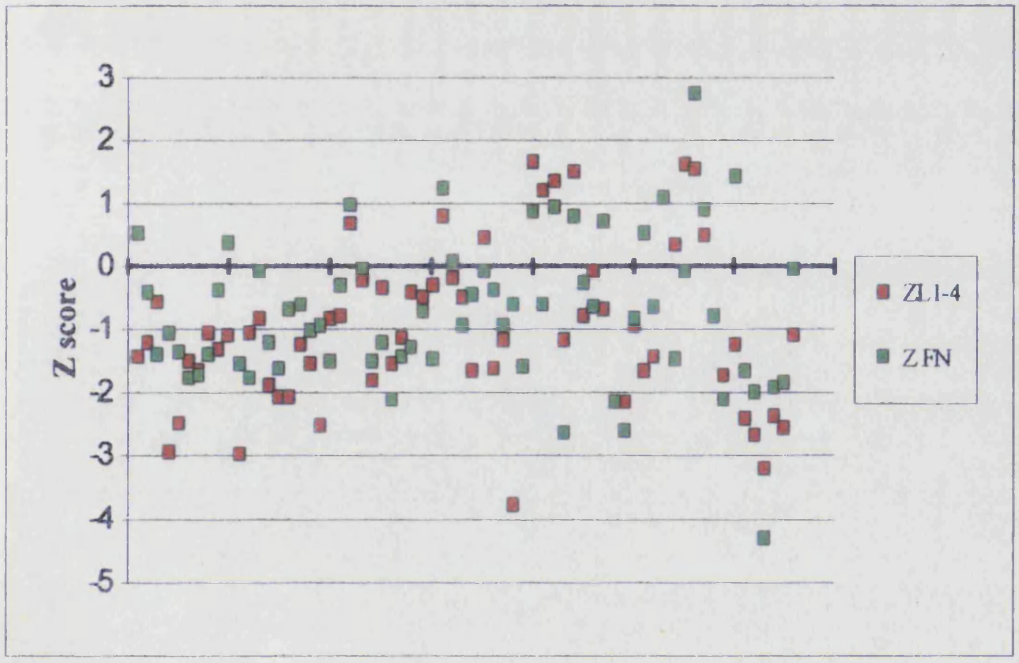


Figure 3. 2. *Z scores of the lumbar spine and femoral neck in patients with AS*



Relationship of BMD with disease duration (Table 3.3)

No significant relationship was observed between BMD of the lumbar spine or femoral neck with the duration of disease in patients with AS (table 3.3).

Table 3. 3. *Relationship of BMD with duration of disease in AS.*

Variable	Disease duration	
	R value	P value
Lumbar spine - BMD (g/cm^2)	-0.07	0.61
Femoral neck - BMD (g/cm^2)	-0.04	0.77
Lumbar spine Z score	-0.04	0.78
Femoral neck Z score	+0.05	0.72

Prevalence of vertebral deformities in AS (Table 3.4)

The prevalence of vertebral deformities in AS using the McCloskey method and UKR was found to be 16.7%. With the same method, using the ASR, the prevalence of deformities increased to 42.4%. With the Eastell method on UKR, the prevalence of grade 1 and grade 2 vertebral deformities was 24.2 and 7.6% respectively and with the ASR, the prevalence increased to 43.9% and 25.8% respectively. The prevalence of deformities is shown in table 3.4.

Table 3. 4. *Prevalence of vertebral deformities in* ***Ankylosing Spondylitis*** *using UKR and ASR analyzed by the Eastell and McCloskey methods.*

Reference values	Method	Prevalence. n = 66	Percentage (%)
UKR	McCloskey	11	16.7
UKR	Eastell	16 - grade1, and 5 - grade 2	24.2 and 7.6
ASR	McCloskey	28	42.4
ASR	Eastell	29 - grade1, and 17- grade 2	43.9 and 25.8

Prevalence of vertebral deformities in the local controls (Table 3.5)

With the McCloskey method, using the UKR, the prevalence of deformities was 2.6% and with the CR, the prevalence increased to 10.2%. With the Eastell method with the UKR, the prevalence of deformities was 5.1% (gradel) and 0% (grade 2), while with the CR, the prevalence increased to 28.2 and 10.2% respectively. The prevalence is given in table 3.5.

Table 3. 5. *Prevalence of deformities in the* ***Local Controls*** *using UKR and CR analyzed by the Eastell and McCloskey methods.*

Reference values	Method	Prevalence. n = 39	Percentage (%)
UKR	McCloskey	1	2.6
UKR	Eastell	2 - grade 1, and 0 - grade 2	5.1 and 0
CR	McCloskey	4	10.2
CR	Eastell	11 - grade 1 and 4-grade 2	28.2 and 10.2

Comparison of prevalence of deformities - AS *Vs Controls (Table 3.6)*

A significantly higher prevalence of vertebral deformities was observed in patients with AS compared with controls using the McCloskey ($p < 0.05$) and Eastell ($p < 0.01$) methods. The UKR were used. The odds ratio for the development of vertebral deformities using the McCloskey and Eastell methods was 5.9 and 6.5 respectively (table 3.6).

Table 3. 6. *Prevalence of deformities in AS compared with controls (Odds Ratio)*

Method used	Odds ratio	95% Confidence Interval
McCloskey	5.9	1.47 - 23.80
Eastell	6.5	1.02 - 41.28

Relationship of vertebral deformities in AS with age and disease duration (Table 3.7)

Using the McCloskey method with the UKR, the relationship between vertebral deformities and age and disease duration was determined. As shown in table 3.7, although individuals with vertebral deformities were older than those without, the difference was not significant ($p = 0.17$). However, individuals with deformities had significantly longer disease duration when compared with the ones without ($p < 0.05$).

Table 3. 7. *Relationship of vertebral deformities in AS with age and disease duration.*

Variable	AS - With deformities	AS - No Deformity	P value
	Mean (SD)	*Mean (SD)*	
Age (years)	41.4 (4.9)	37.8 (7.7)	0.17
Disease duration (years)	12.4 (3.3)	9.3 (5.7)	< 0.05

Type of vertebral deformities in AS patients (Table 3.8)

Using the McCloskey method, based on UKR, vertebral deformities were found in 11 out of 66 patients. The majority of the deformities were biconcave. The type of deformities is shown in table 3.8. The algorithm identified deformities in the following order: crush, wedge, biconcave. Once a deformity was identified in one vertebra, that vertebra was not considered for another deformity. Therefore it was possible to have only one deformity per vertebra.

Table 3. 8. *Type of vertebral deformities in AS defined by McCloskey method using UKR.*

Patient	Crush	Wedge	Biconcave
1	1		1
2			3
3	1		
4			2
5	1		
6		2	
7	1		
8	2		1
9	1		
10			1
11		1	
Total	**7**	**3**	**8**

Note: *It is possible to have only one deformity per vertebra, as the algorithm having identified a vertebra with one type of fracture did not consider it for other types of fractures.*

Site of vertebral deformities (Table 3.9)

95% of the vertebral deformities in patients with AS is found in the thoracic spine with only 5% in the lumbar spine. The distribution of deformities at different vertebral levels is given in table 3.9 and is based on McCloskey's method using UKR. The commonest site for the development of deformities was between T7 and T11, with the maximum number of deformities occurring at T8.

Table 3. 9. *Site of vertebral deformities in AS defined by McCloskey method using UKR.*

Vertebra	Deformity			
	Crush	Wedge	Biconcave	Total
T4		1		1
T5				
T6				
T7	1	1		2
T8	4			4
T9		1	2	3
T10			2	2
T11			3	3
T12	2			2
L1				
L2				
L3				
L4			1	1

Relationship between BMD and vertebral deformities in AS (table 3.10)

No significant relationship was observed between BMD of the lumbar spine or femoral neck with vertebral deformities. Table 3.10 shows the mean BMD values in patients with vertebral deformities as compared to patients without deformities. The deformities were defined by both methods using the UKR.

Table 3. 10. *Comparison of BMD between patients with deformities and those without.*

Variable	Eastell 1 mean (SD)		Eastell 2 mean (SD)		McCloskey mean (SD)	
	AS with Def	AS - No Def	AS with Def	AS - No Def	AS with Def	AS - No Def
BMD(g/cm^2)						
LS	0.97 (0.18)	0.98 (0.12)	0.99 (0.18)	0.98 (0.13)	1.03 (0.15)	0.97 (0.13)
FN	0.86 (0.13)	0.82 (0.11)	0.82 (0.07)	0.83 (0.12)	0.84 (0.12)	0.82 (0.12)
Z score						
LS	-0.94 (1.60)	-0.84 (1.11)	-0.78 (1.70)	-0.87 (1.21)	-0.38 (1.40)	-0.96 (1.20)
FN	-0.29 (1.13)	-0.77 (1.01)	-0.78 (0.57)	-0.64 (1.08)	-0.42 (1.05)	-0.69 (1.05)
T score						
LS	-1.08 (1.67)	-0.93 (1.10)	-0.83 (1.69)	-0.98 (1.23)	-0.51 (1.44)	-1.05 (1.21)
FN	-1.05 (1.23)	-1.43 (1.05)	-1.40 (0.66)	-1.33 (1.13)	-1.23 (1.14)	-1.35 (1.10)

Note: No significant differences were observed.

Def = deformities

Eastell 1 - grade 1 deformities by the Eastell method

Eastell 2 - grade 2 deformities by the Eastell method

Comparison of methods used to determine the prevalence of deformities in AS

The prevalence of deformities determined by various criteria is considerably different. A comparison of the two methods used to define vertebral deformities, using UKR, is shown in table 3.11. The McCloskey method identified 9 of the 16 grade 1 deformities and 4 of the 5 grade 2 deformities defined by the Eastell method.

Table 3. 11. *Comparison between methods used to define vertebral deformities in AS.*

		UK McClos	
		NoDef	*Def*
UK EAST 1	*NoDef*	48	2
	Def	7	9

		UK McClos	
		NoDef	*Def*
UK EAST 2	*NoDef*	54	7
	Def	1	4

Def - Number of vertebrae with deformities

NoDef - Number of vertebrae without deformities

UK EAST 1 - Eastell method (grade1 deformities) with UKR.

UK EAST 2 - Eastell method (grade2 deformities) with UKR.

UK McClos - McCloskey method with UKR.

Discussion

This study shows marked osteoporosis of the lumbar spine and femoral neck in patients with AS compared with age and sex matched controls. The results confirm findings of Will et al (1989), Devogelaer et al (1992) and Mullaji et al (1994) and was based on a greater number of patients with mild disease. The patients chosen had relatively short disease duration and minimal clinical and radiological involvement of the lumbar spine and hip joints to minimise interference with BMD measurement.

In this study the BMD of patients with AS was compared with the normal ranges provided by the manufacturers (Hologic) due to inadequate number of local controls, unlike the above three studies where age and sex matched local controls were used. However, no significant difference between local controls and the normal range of the manufacturers was found in a case control analysis (described in section 2.3) which validated the comparison of the BMD of AS patients with Hologic data.

The reduction in BMD of the lumbar spine can in part be explained by a relative reduction in mobility of the spine due to pain and stiffness. However, the pain and stiffness in AS can be episodic with asymptomatic periods in between. Moreover, this cohort consisted of patients who had normal mobility of their lumbar spines. In addition, all were on regular exercise therapy with the majority being attenders of hospital based in-patient physiotherapy course. Will et al (1989) had observed that patients with AS exercised more than normal controls. This however, cannot explain the reduction of BMD in the femoral neck given that all patients had clinically and radiologically normal hip joints.

The sparing of the appendicular skeleton from the osteoporotic process in mild disease (Will et al 1989, Devogelaer et al 1992, Mullaji et al 1994) suggests that the osteoporosis is mainly due to involvement of the trabecular bone which is metabolically more active and thus susceptible to hormonal and cytokine influences than cortical bone. There is no data on the role of cytokines in causing osteoporosis in AS. Although elevated levels of IL-6 have been demonstrated in AS (Bilgic et al 1994), this did not correlate with disease activity. It is possible that local or systemic release of

cytokines may play a significant role in causing osteoporosis in AS, but more evidence is required before definite conclusions can be drawn.

Evidence for the role of testosterone as a cause of the osteoporosis has been conflicting, with Borbas et al (1986) and Dougados et al (1986) demonstrating increased levels of testosterone, Spector et al (1989) observing no change and Tapia-Serrano et al (1991) demonstrating a diminshed testicular reserve of testosterone compared with normal controls. The relationship of total and biologically active testosterone with BMD and vertebral deformities in patients with AS has been described in the following section.

Low bone formation due to suppression of osteoblastic activity by inflammatory mediators may lead to osteoporosis as well. The following section describes the relationship of markers of bone turnover with BMD and vertebral deformities.

The clinical significance of osteoporosis lies in the development of vertebral fractures. The patients described in this study had a higher prevalence of deformities compared with sex matched healthy controls, in spite of the fact that the controls were older and had fractures themselves. These findings were similar to those of Donnelly et al (1994) who observed an increased prevalence of vertebral fractures in patients with AS compared with controls of similar age range. The higher prevalence of deformities could in part be due to the inclusion of patients who were more susceptible to developing vertebral deformities than other AS patients as they were consecutively selected from outpatient clinics of a hospital. However, no patient had any history of trauma or complained of acute onset back pain. Neither was any patient ever x-rayed to rule out the possibility of vertebral fractures.

An alternative explanation for the increased prevalence could be variation in point placement during morphometry. The UK reference values used here were measured by a different observer at a different location (Berlin) and differences in marking the vertebrae were quite likely to occur. In addition, reference values can vary between populations (O'Neill et al 1994). In this study external reference values were applied

which may in part explain the high prevalence of deformities. This however, would not influence the case control analyses given that all subjects were exposed to the same set of reference values. Ideally the source of reference values should be from within the population itself, but due to the small sample size of the populations being studied (AS patients and control subjects), external reference values were used. Compounding this problem is the absence of a "gold standard" for defining vertebral deformities which adds to the differences in the prevalence observed by different methods.

The two methods used here (Eastell and McCloskey) differ considerably in the way in which they define vertebral deformities (section 2.5). The McCloskey method uses more stringent criteria for the definition of deformities; two separate criteria being used to define a deformity compared with only one criteria used by the Eastell method. There is also considerable difference in the sensitivity and specificity of the two methods. The Eastell method has been described to have a higher number of false positives (12%) as compared to the McCloskey method (1%) when 3 SD is used as a cutoff for the determination of vertebral deformities.

These patients did not have a history of trauma, which suggests that the deformities occurred spontaneously. This implicates osteoporosis as a causative factor for deformity (Ralston et al 1990). However, in this study no relationship was evident between BMD of either the lumbar spine or femoral neck with the deformities. This result was in consensus with the findings of Donnelly et al (1994). However, in their cohort of patients almost half had advanced radiographic changes in the spine. The lack of relationship between BMD of the *lumbar spine* and vertebral deformities in AS may therefore be partly due to the fact that the measurement of antero-posterior (AP) BMD in the lumbar spine includes cortical and trabecular bone while the osteoporosis in AS involves trabecular bone only. Lateral spine densitometry should have greater diagnostic sensitivity for osteopaenia as selective measurement of trabecular bone is possible. Devogelaer et al (1992) observed osteopaenia of vertebral bodies by QCT despite the presence of extensive syndesmophytes.

Densitometry of the lateral spine was not available for this study as the BMD was measured by a Hologic QDR 1000. Newer dual energy x-ray absorptiometers (Hologic QDR 2000, 4500 and Lunar Expert) are becoming available wherein selective BMD and morphometry of the lateral vertebrae is possible.

An alternative explanation for the lack of relationship between BMD of the *lumbar spine* and vertebral deformities could be due to the inclusion of individuals with higher BMD. This may be a reflection of increased bone formation which was not apparent in ordinary radiographs. Certainly, the radiographs of these patients did not reveal any obvious syndesmophytes in the thoracic or lumbar spines.

The lack of correlation of *femoral neck* BMD with vertebral deformities may be due to the fact that BMD measurements are site specific. For example, BMD of the lumbar spine or distal radius does not predict the risk of hip fracture as well as the BMD of the femoral neck (Cummings et al 1993).

A further reason for the lack of relationship between BMD overall and vertebral deformities may be that not all changes are due to osteoporosis and that some other factors may in addition be responsible for the alteration in vertebral shape. An analysis of radiographs of patients in this study suggests that conditions like spondylodiscitis and Romanus lesions may account for the disordered architecture of the vertebral bodies in AS. It is also possible that developmental differences in bone architecture may be responsible for differences in BMD of individual vertebrae, which may in turn be responsible for an overall lack of relationship between BMD and vertebral deformities. A lack of relationship between BMD and vertebral deformities has also been observed in patients with rheumatoid arthritis (Spector et al 1993).

This study highlights the importance of vertebral morphometry and radiographic assessment of the spine being used to complement each other (Raymakers et al 1990). Although radiographic assessment of bone x-rays may be an insensitive method for the diagnosis of vertebral deformities, morphometric assessment is compounded by the absence of a gold standard for the definition of vertebral fractures. The fact that

conditions like Scheurmann's disease, epiphyseal dysplasia of the spine and, in this study, spondylodiscitis and Romanus lesions account for some of the vertebral deformities, emphasizes the need for morphometry being complemented by visual assessment of spinal deformities.

The results of this study suggest that the risk of developing vertebral deformities increases with age and disease duration. Although similar observations were made by Donnelly et al (1994) and Cooper et al (1994), the cohort of patients in the latter two studies included individuals with advanced spinal changes. In contrast the patients in this study were characterised by absent or incipient syndesmophytes. The fact that these patients sustained vertebral deformities in the absence of advanced spinal changes suggests that the disease process may have a role in the development of deformities before immobility has occurred. Will et al (1989) in their study had suggested that the osteoporosis in AS could well be a primary pathologic event. The development of deformities in early disease may in part be responsible for the dorsal kyphosis seen in advanced disease.

In conclusion, this study confirms the presence of axial osteoporosis and vertebral deformities in patients with mild AS. The lack of relationship between BMD and vertebral deformity suggests that other factors in addition to low bone density are perhaps implicated in spinal deformation. The propensity for these deformities to increase with advancing disease, and their presence in the thoracic spine may in part be responsible for the kyphotic posture seen in advanced ankylosing spondylitis.

CHAPTER 4 - BIOCHEMICAL MARKERS OF BONE TURNOVER IN MILD ANKYLOSING SPONDYLITIS AND THEIR RELATIONSHIP WITH BONE MINERAL DENSITY AND VERTEBRAL DEFORMITIES

Summary

Background and aims

The development of osteoporosis is usually due to changes in bone formation and resorption. This can be quantified biochemically by measuring markers of bone formation and resorption, and radiographically by recording BMD. The clinical importance of osteoporosis lies in the development of vertebral fractures, which can be quantified by morphometry. The aim of this study was to measure biochemical markers of bone turnover in patients with mild AS and to evaluate their relationship with BMD and vertebral deformities.

Methods

The biochemical markers of bone turnover were measured as described in section 2.6, and BMD and vertebral morphometry were recorded as described in sections 2.7 and 2.5 respectively. Differences in the biochemical and hormonal markers of bone turnover were evaluated by dividing the AS patients into quintiles based on BMD, quintile1 being the group with the lowest BMD and quintile 5 the highest. Similar comparison of markers were performed between patients who had vertebral deformities and those without.

Results

Patients with AS had significantly lower mean osteocalcin [9.03 (2.7) μg/l] compared with controls of similar age range [11.05 (2.3) μg/l, ($p < 0.001$)] and higher mean values of total and bone alkaline phosphatase [73.09 (19.5) U/l *Vs* 53.02 (16.6) U/l and 38.54 (9.9) U/l *Vs* 30.35 (8.2) U/l respectively, ($p < 0.001$)]. The 25 hydroxy vitamin D and parathyroid hormone (PTH) levels were slightly but not significantly higher in patients with AS. There was no significant difference in Dpyr and Pyr between patients

and controls. There was a significant relationship of the biochemical markers of bone turnover with acute phase reactants ($p < 0.01$), but not with vertebral deformities or BMD. However, patients with the lowest spinal BMD (quintile1) had a trend towards lower osteocalcin, bone alkaline phosphatase (BALP), bone sialoprotein (BSP), testosterone free index (TFI) and higher acute phase reactants.

Conclusions

These results suggest that patients with AS have osteoporosis characterised by low bone formation which is most likely due to a suppression of osteoblastic activity by inflammatory mediators; bone resorption tends to remain unaffected. Low bone mass may be aggravated by a state of relative hypogonadism that exists in a subgroup of male AS patients characterised by very low spinal BMD. No relationship was observed between markers of bone turnover, BMD and vertebral deformities. There is a disparity between osteocalcin and alkaline phosphatase which needs further evaluation.

Introduction

Osteoporosis can be defined as a disease characterized by low bone mass and microarchitectural deterioration of bone tissue, leading to enhanced bone fragility and a consequent increase in fracture risk. Under normal circumstances a tight coupling between bone formation and resorption exists. Uncoupling of this well regulated mechanism leads to a change in the rate of bone formation or resorption which is reflected in a change in markers of bone turnover.

AS is characterised by low as well as increased bone formation. The decreased bone formation may result in osteoporosis and the increased bone formation contributes to the syndesmophytosis and eventual ankylosis seen in advanced disease. Franck et al (1993) observed low osteocalcin levels in AS. Ekenstam et al (1986) found similar results in RA and seronegative spondyloarthropathies and suggested that the decrease in osteocalcin was as a result of inflammation. Neither study however demonstrated any relationship between markers of bone turnover and serological indices of inflammation.

There is no published data on the relationship of biochemical markers of bone metabolism with BMD and vertebral deformities in AS. There is no data on the state of bone resorption in AS.

The *aims* of this study were to assess biochemical markers of bone metabolism in patients with mild AS and to determine their relationship with BMD and vertebral deformity.

Methods

Selection of patients

Of the 66 patients described in the previous study (chapter 3), a cohort of 56 patients were enrolled for this study (10 patients declined to participate). The selection of patients has been described in section 2.2.

Selection of controls

The selection of controls for this study was as described in section 2.3.

Clinical assessment of disease activity

Clinical assessments to evaluate the disease activity in the patients with AS were carried out as detailed in chapter 2.4. The acute phase reactants measured included plasma viscosity and C-reactive protein (CRP).

Laboratory investigations

Routine investigations

The following haematological and biochemical tests were carried out as routine:

- FBC (including white cell count and platelet count),
- Acute phase reactants (plasma viscosity and C-reactive protein),
- Renal function (creatinine, urea and electrolytes),
- Liver function (bilirubin, alkaline phosphatase, γGT, ALT and AST),
- Corrected calcium, phosphate and albumin,
- Thyroid function (TSH),
- Serum protein electrophoresis,
- Sex hormone profile (FSH, LH, Prolactin, Testosterone, SHBG),
- 25 hydroxy-vitamin D [25 (OH) Vit D] and parathyroid hormone (PTH),
- 2 hour urinary calcium/creatinine ratio,
- 24 hour urinary calcium and cortisol,

Biochemical markers of bone turnover

The following tests were performed:

- Serum osteocalcin,
- Total alkaline phosphatase (ALP) and bone alkaline phosphatase (BALP),
- Serum bone sialoprotein (BSP),
- Urinary deoxypyridinoline (Dpyr) and pyridinoline (Pyr)

All results are expressed as mean (SD) unless specified otherwise.

Radiographic assessment

Radiographs and BMD were recorded as described in sections 2.4 and 2.7 respectively. The time difference between the radiographs and BMD was less than three months in all cases.

Defining the fracture group

Vertebral deformities were defined by the Eastell and McCloskey methods using UK reference values (described in section 2.5).

Identifying BMD quintiles

Patients with AS were divided into quintiles based on BMD of the lumbar spine and femoral neck. Acute phase reactants and markers of bone turnover were compared between individuals with the lowest and highest BMD (quintiles 1 and 5 respectively).

Statistical analysis

Statistical analyses were performed using the Mann Whitney tests, the Wilcoxon Rank sum tests and separate variance t-tests. Relationships were calculated using Spearman's correlation coefficients. Variables were considered to be significant if the p value was < 0.05. For variables that were not normally distributed, logarithmic transformations were carried out and geometric means and standard deviations were calculated.

Results

Baseline descriptive characteristics of all patients enrolled in the study are shown in tables 4.1.a, 4.1.b, and 4.1.c.

Table 4.1.a shows the mean age of the patients was 38.39 years (7.4) and the mean disease duration was 9.85 years (5.5). The average body mass index was 25.25 (3.1). The average early morning stiffness was 1 hour 5 minutes and the lumbar spine mobility was normal [mean modified Schober's 6.93 (1.4) cms].

Table 4. 1. a. *Baseline demographic and clinical features of patients with AS.*

Variable	Mean	Standard Deviation
Demographic features		
Age (years)	38.39	7.4
Duration (years)	9.85	5.5
Height (m)	1.77	0.0
Weight (kg)	79.23	10.7
Body mass index [weight(kg)/height(m^2)]	25.25	3.1
Clinical features		
VAS-Cervical spine (cm)	3.16	2.6
VAS-Thoracic spine (cm)	2.12	1.9
VAS-Lumbar spine (cm)	3.51	2.5
Early morning stiffness (mins)	65	11.1
Metrology		
Tragus-Wall (cms)	10.09	2.4
Chest Expansion (cms)	4.73	1.4
Modified Schober's (cms)	6.93	1.4
Intermalleolar distance (cms)	115.08	15.3

Table 4.1.b. contains the mean haematological, immunological and biochemical details. The acute phase reactants of inflammation were abnormal, mean plasma viscosity 1.80 (0.1) mPas and mean CRP 0.02 (0) g/l. The mean corrected calcium [2.28 (0) mmol/l] and phosphate [1.05 (0.1) mmol/l] were in the normal range as were the 25 (OH) Vit D [22.25 (10.4)] and PTH [3.77 (2.7)mmol/l]. No patient had abnormal levels of 24 hour urinary calcium [5.15 (2.2) mmol/24 hours] and urinary free cortisol [133.58 (64.1) nmol/24 hours].

Table 4. 1. b. *Baseline laboratory investigations in patients with AS.*

Variable	Mean	Standard deviation
Acute phase reactants		
Plasma Viscosity (mPas)	1.80	0.1
C-Reactive Protein (g/l)	0.02	0.0
Biochemistry		
Creatinine (mmol/l)	78.39	11.8
Corrected Calcium (mmol/l)	2.28	0.0
Phosphate (mmol/l)	1.05	0.1
25 Hydroxyvitamin D (μmol/l)	22.25	10.4
Parathyroid hormone (pmol/l)	3.77	2.7
Urine calcium/creatinine(mmol/l)	0.29	0.2
24 hr urine calcium (mmol/24hrs)	5.15	2.2
Free urinary cortisol (nmol/24hrs)	133.58	64.1
Thyroid Stimulating Hormone (mU/l)	1.52	0.6
Sex hormones		
Follicle Stimulating Hormone (IU/L)	5.38	3.9
Luteinizing Hormone (IU/L)	3.4	1.7
Testosterone (nmol/l)	16.02	5.0
Sex hormone binding globulin (nmol/l)	27.29	10.6
Testosterone Free Index (TFI)	63.87	23.2
Biochemical markers of bone turnover		
Alkaline Phosphatase (U/l)	73.09	19.5
Bone Alkaline Phosphatase (U/l)	38.54	9.9
Osteocalcin (μg/l)	9.03	2.7
Bone sialoprotein (ng/l)	81.52	20.4
Pyr/Creatinine (nmol/mmol)	15.66	10.8
Dpyr/Creatinine (nmol/mmol)	4.89	3.9
Pyr/Dpyr	3.2	1.4

Patients with AS were characterised by the radiographic absence of syndesmophytes from their lumbar and thoracic spines, and, normal hip joints. The mean Z scores of the lumbar spine and femoral neck were [-1.01 (1.2) and -0.73 (1.1)] respectively (table 4.1.c.).

Table 4. 1. c. *Baseline radiographic details of patients with AS*

Variable	Mean	Standard deviation
Radiographic scoring		
Xray-SI Joint-Right	2.86	1.0
Xray-SI Joint-Left	2.84	1.0
Xray-Lumbar spine	0.61	0.5
Xray-Thoracic spine	0.45	0.5
Xray-Cervical spine	0.16	0.6
Xray-right hip joint	0.03	0.1
Xray-left hip joint	0.01	0.1
Bone mineral density		
BMD Lumbar spine (gm/cm^2)	0.98	0.1
BMD Femoral neck (gm/cm^2)	0.83	0.1
Z score Lumbar spine (L1-4)	-1.01	1.2
Z score Femoral neck	-0.73	1.1
T score Lumbar spine (L1-4)	-1.14	1.2
T score Femoral neck	-1.44	1.2

Laboratory investigations

All patients had normal renal and liver functions. The mean corrected calcium and phosphate were 2.28 mmol/l (0.1) and 1.05 mmol/l (0.1) respectively. The 24 hour urinary calcium and free urinary cortisol levels were within normal limits in all patients. The mean urinary calcium/creatinine ratio was 0.28 mmol/l (normal < 0.3).

Markers of bone turnover: case control analyses and relationships.

Patients with AS had significantly lower mean serum osteocalcin compared with controls ($p < 0.001$) (fig 4.1), and significantly higher mean levels of ALP and BALP ($p < 0.001$) (fig 4.2). There was no significant difference in BSP between patients and controls [81.52 (20.3) ng/ml *Vs* 76.7 (11.3) ng/ml]. Excretion of Dpyr and Pyr in patients with AS was not significantly different from that in controls [4.90 (3.9) nmol/mmol *Vs* 4.00 (3.6) nmol/mmol, and 15.66 (10.8) nmol/mmol *Vs* 12.24 (10.3) nmol/mmol respectively] (fig 4.3). In addition, there was no significant difference in the ratio of Dpyr/Pyr between patients and controls (3.20 *Vs* 3.05 respectively).

Figure 4. 1. *Osteocalcin: a case control analysis (* * = $p < 0.001$).*

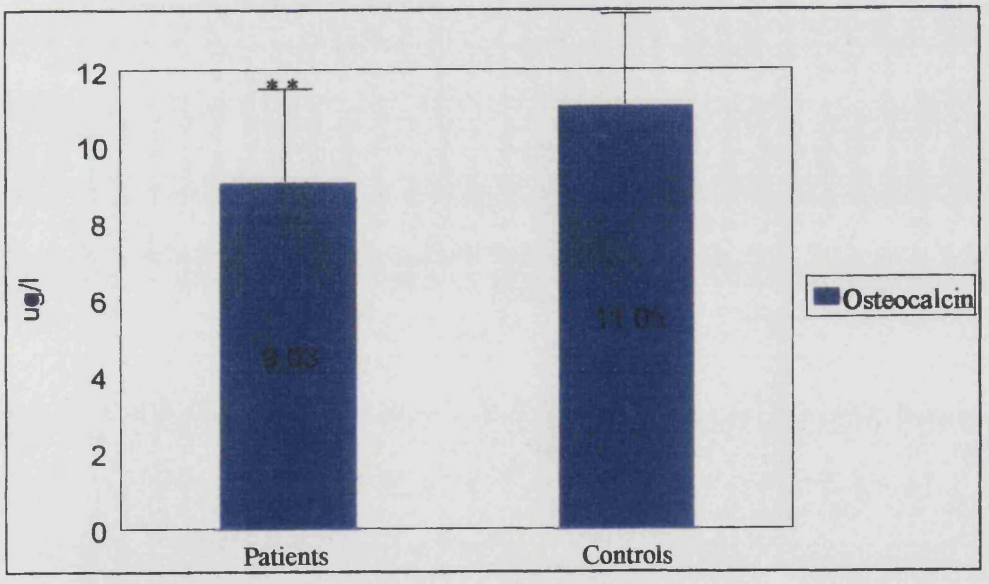


Figure 4. 2. *Alkaline phosphatase (ALP) & Bone alkaline phosphatase (BALP): case control analysis (** = $p < 0.001$).*

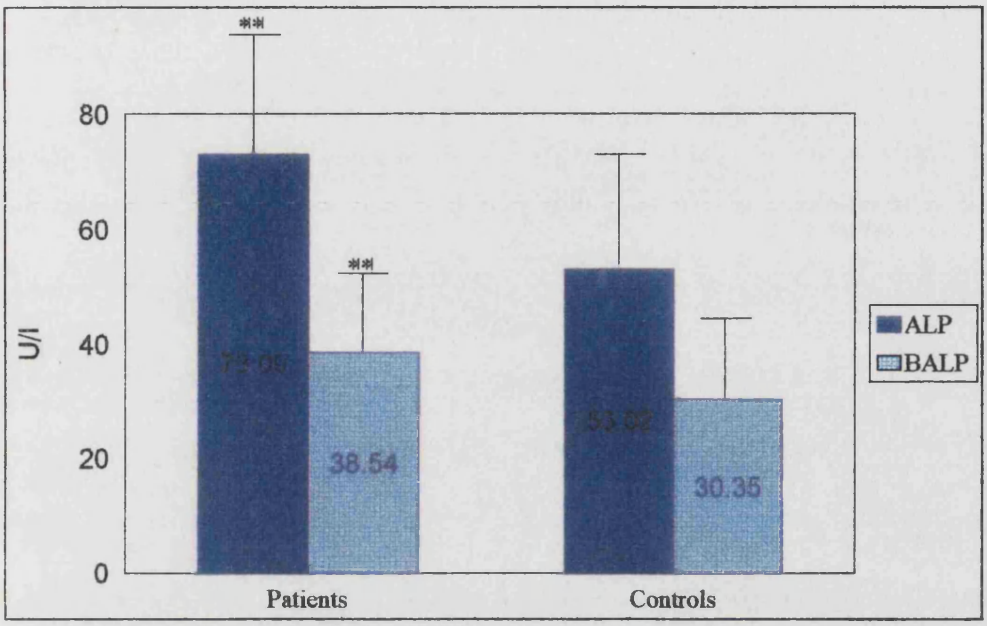
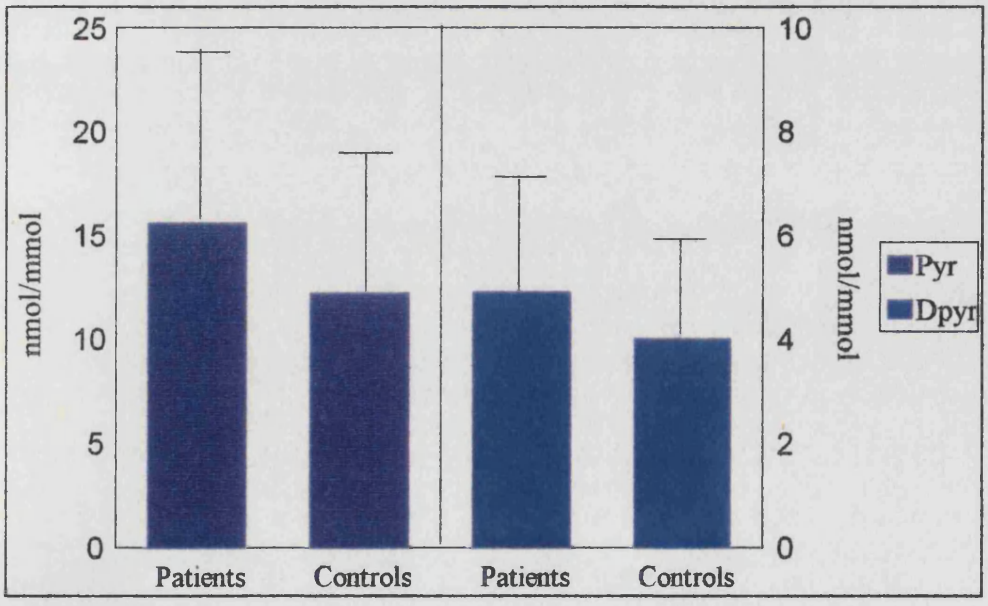


Figure 4. 3. *Comparison of Dpyr and Pyr between patients and controls.*



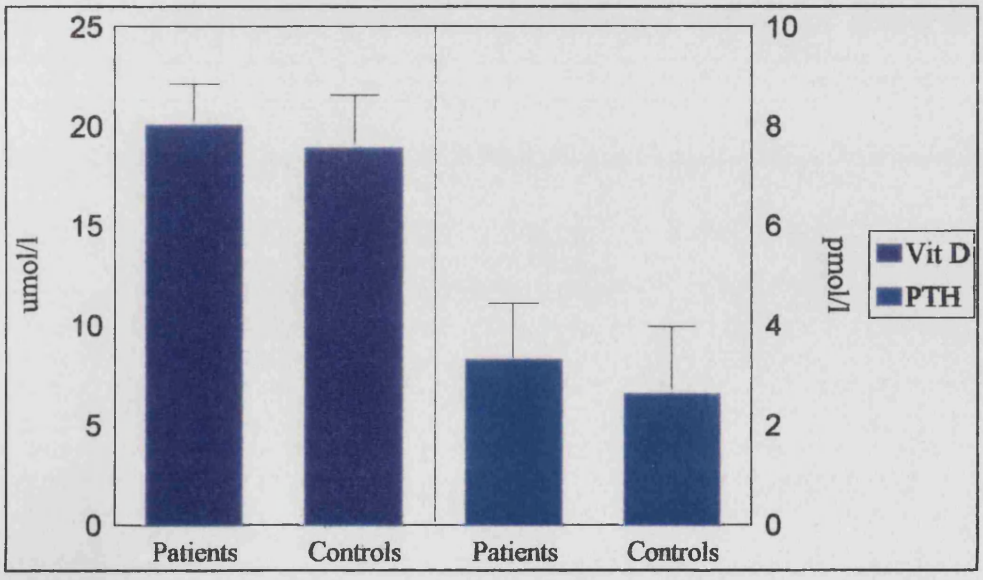
Significant but weak relationships of BALP were seen with osteocalcin, BSP, Dpyr and Pyr (table 4.2). ALP and BALP related strongly with each other (r = 0.87, p<0.001).

Table 4. 2. *Relationship of BALP with other markers of bone turnover.*

	Osteocalcin		BSP		Dpyr		Pyr	
	R value	P value	R value	P value	R value	P value	R value	P value
BALP	0.26	< 0.05	0.36	< 0.01	0.27	< 0.05	0.25	< 0.05

Although mean 25 (OH) Vit D and PTH levels were higher in patients with AS compared with controls, the differences were not significant [20.03 (1.6) µmol/l *Vs* 18.9 (2.9) µmol/l and 3.31 (1.5) pmol/l *Vs* 2.63 (0.4) pmol/l respectively] (fig 4.4). There was a significant relationship of urinary calcium/creatinine ratio with 25 (OH) Vit D (r = 0.26, p < 0.05) and PTH (r = 0.7, p < 0.001). In addition, PTH correlated significantly with Dpyr (r = 0.3, p < 0.05) and Pyr (r = 0.28, p < 0.05).

Figure 4. 4. *Vitamin D and PTH: comparison between patients & controls.*



There was no significant difference in the mean TFI between patients and controls [63.8 (23.18) nmol *Vs* 60 (22.15) nmol] and neither were there any differences in the levels of follicle stimulating hormone (FSH) and luteinizing hormone (LH). A weak but significant relationship was found between testosterone and serum osteocalcin ($r = 0.3$, $p < 0.05$).

Relationship between markers of bone turnover and disease activity.

The biochemical and hormone markers did not correlate with any symptom of AS. However, the acute phase reactants did demonstrate a significant relationship with markers of bone formation and resorption (table 4.3).

Table 4. 3. *Correlations between acute phase reactants and markers of bone turnover.*

	ALP		BALP		Dpyr		Pyr		Osteocalcin	
	R value	P value	R value	P value	R value	P value	R value	P value	R value	P value
P.Viscosity	0.47	< 0.001	0.30	< 0.02	0.31	< 0.01	0.32	< 0.01	-0.3	< 0.03
CRP	0.46	< 0.01	0.31	< 0.02	0.36	< 0.01	0.35	< 0.01	-0.3	< 0.05

Relationship between markers of bone turnover and BMD

Using Spearman's correlation coefficients no significant relation between any marker of bone turnover and BMD of either the lumbar spine or femoral neck was observed. However, the patients with the lowest lumbar spine BMD (quintile 1) had significantly lower TFI and higher SHBG compared with the patients with the highest BMD (quintile 5) (table 4.4). A similar decrease in TFI and increase in SHBG was evident in the patients with the lowest femoral neck BMD as well, but the changes were not significant. In addition, patients with the lowest lumbar spine BMD had a trend towards higher plasma viscosity and lower osteocalcin, alkaline phosphatase, BALP, and BSP.

Table 4. 4. *Comparison of acute phase reactants and markers of bone turnover between patients with the lowest and highest BMD.*

Variable	Lum spine Quintile1	Lum spine Quintile 5		Fem neck Quintile 1	Fem neck Quintile 5	
	Mean (SD)	*Mean (SD)*	*P value*	*Mean (SD)*	*Mean (SD)*	*P value*
Plasma Visc	1.79 (0.1)	1.76 (0.1)	NS	1.77 (0.1)	1.75 (0.1)	NS
CRP	0.02 (0.01)	0.02 (0.01)	NS	0.02 (0.02)	0.01 (0.01)	NS
Testosterone	15.1 (4.4)	16.8 (3.3)	NS	15.2 (6.3)	16.8 (4.8)	NS
SHBG	***31.4 (9.1)***	***23.7 (5.7)***	***<0.05***	27.3 (4.1)	26.6 (8.3)	NS
TFI	***49.3 (9.9)***	***73.4 (15.1)***	***<0.001***	54.9 (18.2)	68 (22.9)	NS
25(OH)VitD	21.8 (10)	21.6 (7.50)	NS	19.3 (12.7)	25.7 (8.7)	NS
PTH	3.7 (0.9)	3.3 (0.9)	NS	4.4 (2.4)	3.4 (1)	NS
Ur Cal/creat	0.2 (0.1)	0.3 (0.1)	NS	0.1 (0.1)	0.3 (0.1)	NS
ALP	71.9 (16.5)	76.9 (24.1)	NS	77.1 (14.1)	74.4 (21.8)	NS
BALP	37.4 (8.9)	42.2 (11.9)	NS	41 (8)	39.6 (11.7)	NS
Osteocalcin	8.9 (2.4)	9.8 (2.7)	NS	9.8 (1.7)	8.1 (3.1)	NS
BSP	72.4 (12.7)	83.3 (14.6)	NS	79.1 (15.8)	84.6 (10.3)	NS
Pyr/creat	23.3 (15.8)	15.1 (8.6)	NS	12.9 (5.9)	13.9 (9.7)	NS
Dpyr/creat	4.6 (3.2)	5.3 (4.3)	NS	6.9 (6.4)	3.6 (2.5)	NS

Relationship between markers of bone turnover and vertebral deformities

The relationship between vertebral deformities and markers of bone turnover in AS patients is described in table 4.5. Osteocalcin was reduced on the whole in patients with vertebral deformities, significantly so ($p < 0.01$) in patients with the severest deformities (4 SD below the normal mean). TFI was also reduced in the patients with deformities on the whole but the reduction was not significant.

Table 4. 5. *Comparison of markers of bone turnover between patients with vertebral deformities and those without.*

Variable	Eastell - 1			Eastell - 2			McCloskey		
	AS with D	**AS - No D**		**AS with D**	**AS - No D**		**AS with D**	**AS - No D**	
	Mean (SD)	*Mean (SD)*	*P*	*Mean (SD)*	*Mean (SD)*	*P*	*Mean (SD)*	*Mean (SD)*	*P*
Testosterone	16.3 (4.6)	15.9 (5.2)	NS	17.3 (4)	15.9 (5.1)	NS	16.7 (5.5)	15.9 (5)	NS
SHBG	29.5 (8.5)	26.5 (11.2)	NS	31 (10.7)	27 (10.7)	NS	29.3 (8.9)	26.9 (11)	NS
Vit D	23.5 (11.6)	21.8 (10.2)	NS	31 (15.8)	21.6 (9.8)	NS	30.4 (13.6)	20.7 (9.1)	NS
TFI	60.1 (24.9)	65.1 (22.7)	NS	61.1 (25.7)	64.1 (23)	NS	60.2 (21.5)	64.6 (23.6)	NS
PTH	3.7 (8.04)	3.8 (3)	NS	3.1 (1)	3.8 (2.8)	NS	3 (0.9)	3.9 (2.9)	NS
Osteocalcin	8 (3.2)	9.4 (2.5)	NS	***6.7 (0.6)***	***9.2 (2.7)***	***<0.01***	7.7 (2.9)	9.3 (2.6)	NS
BALP	38 (7.8)	38.7 (10.6)	NS	37 (9)	38.7 (10.1)	NS	39.3 (10.3)	38.4 (10)	NS
BSP	86.9 (33.3)	79.7 (13.9)	NS	83.9 (25)	81.3 (20.3)	NS	93.8 (38.9)	79.2 (14.1)	NS
Ur Cal/creat	0.3 (0.2)	0.3 (0.3)	NS	0.4 (0.3)	0.3 (0.3)	NS	0.3 (0.2)	0.3 (0.3)	NS
Pyr/creat	20.6 (13.1)	14 (9.5)	NS	12.2 (8.4)	15.9 (11)	NS	19.2 (13.6)	15 (10.2)	NS
Dpyr/creat	6.5 (4.9)	4.3 (3.5)	NS	6.1 (7)	4.8 (3.7)	NS	7 (5.5)	4.5 (3.5)	NS

D - deformity

Deformities defined using UKR

NS - not significant

DISCUSSION

The low mean osteocalcin level seen in the cohort of AS patients in this study is in consensus with findings of Franck et al (1993) and Ekenstam et al (1986). As osteocalcin is considered to be a marker for bone formation and is expressed primarily by osteoblasts, the low level suggests reduced rate of bone formation. Suppression of bone formation in these patients is most likely to be as a result of reduced osteoblastic activity. It is possible that the osteoblasts are suppressed by inflammatory mediators, as a strong relationship is seen between the acute phase reactants of inflammation (plasma viscosity and CRP) and markers of bone turnover (osteocalcin, alkaline phosphatase, BALP and urinary crosslinks). Ekenstam et al (1986) suggested that the reduction in osteocalcin was due to the effect of inflammatory factors on osteoblasts. They based their conclusions on the fact that the reduced osteocalcin level increased concomitantly with reduction of synovitis in patients with RA following treatment with chloroquine and D-penicillamine. The relationship between inflammatory mediators and osteoblastic activity is further strengthened by the fact that osteocalcin levels have been reported to be normal in chronic diseases of non-inflammatory origin (Slovik et al 1984). In addition, the levels of 25 (OH) Vit D and PTH were slightly, but not significantly, higher in the patients with AS compared with controls. As these two calciotropic hormones regulate the synthesis of osteocalcin, they are unlikely to be responsible for the low osteocalcin level seen in the patients of this study. A similar increase in levels of these calciotropic hormones in patients with AS have been reported in previous studies as well (Fairney et al 1975, Franck et al 1993).

The significant relationship observed between ALP and BALP suggests that the high ALP was due to an increase in the bone fraction of its isoenzyme. This is in consensus with findings of Kendall et al (1973), but contradicts the findings of Sheehan et al (1988) who felt that the increase in ALP in AS was due to the hepatic isoenzyme. The increase in ALP in the present study is unlikely to be due to hepatic involvement, as all individuals had normal hepatic functions, including serum levels of gamma glutamyl

transpeptidase, alanine transaminase and aspartate transaminase. The high mean levels of ALP and BALP in the cohort were possibly due to the inclusion of individuals who had increased bone formation which was not evident in ordinary radiographs. As a significant amount of disease activity in AS occurs at the entheses, it is possible that the high ALP and BALP are a reflection of increased osteoblastic activity at these sites. The low level of osteocalcin, ALP and BALP in the subgroup of patients with the lowest spinal BMD compared with patients with normal BMD may well be due to significantly reduced osteoblastic activity in individuals characterised by severe osteoporosis.

It is possible that the differences seen between osteocalcin, ALP and BALP could well be due to differences in the way these markers of bone turnover were assayed in the laboratory. Newer ELISA techniques using monoclonal antibodies for their detection may well improve the sensitivity, and subtle differences may then become evident. This disparity between alkaline phosphatase and osteocalcin in AS has been reported in previous studies as well (Franck et al 1993), and needs further evaluation.

Bone resorption was unaffected in this cohort of patients with AS, with no significant difference in levels of Pyr and Dpyr between patients and controls. The ratio of Pyr to Dpyr was also not significantly elevated. However, the significant relationship between acute phase reactants of inflammation and Pyr and Dpyr observed in this study suggests that bone resorption has the propensity to be involved by disease activity in AS. A possible explanation for the pyridinium levels not altering significantly in this study could be the fact that the cohort of patients studied here had mild disease. A repeat study with patients with more advanced disease would help to clarify this relationship further.

These results suggest that the osteoporosis in mild AS is primarily as a result of reduction in bone formation rates. In contrast, patients with RA have been shown to have higher levels of Pyr and Dpyr (Black et al 1989, Seibel et al 1989) and lower

osteocalcin (Ekenstam et al 1986) compared with normal controls. This difference between the two inflammatory arthritides suggests that the pathogenesis for osteoporosis may be different in each condition.

The reduction of TFI in patients with the lowest spinal BMD suggests that low testosterone levels could constitute at least a partial explanation for the low bone mass encountered in men with AS. The significant relationship observed between osteocalcin and testosterone indicates that low testosterone levels contribute towards decreasing the bone mass further in the subgroup of men with AS who have low bone formation. TFI is a measure of free or biologically available testosterone and is a more sensitive parameter than total testosterone. The cause of the depressed TFI in the present study was unlikely to be due to hypothalamic causes as FSH and LH levels were within normal limits. A diminished testicular reserve in AS was reported by Tapia-Serrano et al (1991), but Borbas et al (1986) and Dougados et al (1986) reported high levels in AS patients. Spector et al (1988) could find no significant difference between the serum testosterone levels of AS patients and control subjects, but did describe a significant reduction in levels of total and free testosterone in 141 patients with rheumatoid arthritis (RA). Neither of the four studies mentioned described the relationship of sex hormones with bone mass (measured quantitatively) in AS. Histological studies of hypogonadal osteoporosis in men demonstrated an increase in bone resorption and decrease in mineralization (Francis et al 1986). They also observed a reduction in both trabecular and cortical bone mass. The patients with AS in this study did not have increased resorption and the cortical bone mass in AS has been shown to be normal (Will et al 1989, Devogelaer et al 1992, Mullaji et al 1994). Thus the relationship between sex hormones and bone mass needs further evaluation.

The lack of a relationship between the markers of bone turnover and BMD and vertebral fractures is most likely due to the fact that the markers of bone turnover reflect events at the time when the test is performed whereas changes in BMD and vertebral deformities occur due to changes in bone turnover that occur over a period of time. The relationships between the markers, BMD and vertebral deformities have

not proven to be very useful except in population studies. There was however, a trend towards lower osteocalcin and TFI in patients with deformities compared with those without. In addition, serum osteocalcin levels did relate significantly with the vertebral deformities that were 4 SD below the mean (grade 2 deformities by the Eastell method). In view of this latter observation it is possible that a more significant relationship of osteocalcin and TFI with vertebral deformities that were between 3-4 SD below the mean would have been evident had the number of patients been greater.

In conclusion, patients with AS have low bone formation which is most likely due to a suppression of osteoblastic activity by inflammatory factors. A state of relative hypogonadism exists in a subgroup of male patients characterised by very low spinal BMD which may contribute to decreasing the bone mass even further. Resorption appears to be unaffected in patients with AS who have mild disease. Biochemical markers of bone turnover do not relate significantly with osteoporosis and vertebral deformities in mild AS. However the potential relationship of osteocalcin and TFI with vertebral deformities needs to be evaluated further with a greater number of patients.

CHAPTER 5 - THE EFFICACY OF ETIDRONATE IN MALES WITH MILD ANKYLOSING SPONDYLITIS AND OSTEOPOROSIS: A DOUBLE BLIND PLACEBO CONTROLLED STUDY

Summary

Background and aims

Osteoporosis and vertebral fractures are complications of mild as well as advanced AS. It is possible that this is eventually responsible for the characteristic kyphotic deformity of the spine seen in cases of advanced AS. The aim of this study was to assess the efficacy of etidronate on the disease activity, markers of bone metabolism and BMD in patients with mild AS and osteoporosis.

Methods

All patients enrolled in the study had mild AS with osteoporosis and radiographically normal spines and hips. Disease activity and biochemical and hormonal markers of bone metabolism were assessed in all patients. All individuals had x-rays of the whole spine, pelvis and hip joints at entry and at the end of the study. BMD was performed in all individuals at 0 weeks, after 32 weeks and at the end of the study (65 weeks). The patients were randomly assigned to two groups, viz. placebo and etidronate, and the medications were administered in an intermittent and cyclical manner as per standard protocol.

Results

Following completion of the study, individuals in the etidronate group had a significant improvement in their symptoms and BMD of the lumbar spine. There was no change however, in their acute phase reactants or markers of bone turnover. There was no significant difference between any variable when the two groups were compared at the end of the study.

Conclusions

Although etidronate has a beneficial effect on symptoms and lumbar spine BMD in patients with AS who have osteoporosis, this needs further validation. It has no demonstrable effect on the serological indices of inflammation and markers of bone turnover.

Introduction

AS is a unique disease in which simultaneous bone loss and new bone formation occurs. In the previous section it was shown that AS is associated with low bone formation. Although the presence of osteoporosis and verterbal fractures in AS has been demonstrated, there are no reports on the treatment of these complications. The pain and stiffness in the spine in AS are due to inflammatory disease activity, but some of the spinal symptoms may be due to microfractures of the vertebral bodies which may occur as a result of osteoporosis.

In this study, the effects of the bisphosphonate etidronate on the osteoporosis in patients with mild AS have been evaluated. The therapeutic efficacy of etidronate in postmenopausal osteoporosis is well documented (Storm et al 1990, Watts et al 1990). Both studies observed a significant increase in the BMD of the spine and reductions in vertebral fracture rates over a two year period. The benefit was found to be greatest in the women who had the lowest BMD.

The long term (5 years) benefits of etidronate in the treatment of osteoporosis are now well recognised (Steiniche et al 1993, Papapoulos et al 1993), the former study utilizing histomorphometry in osteoporotic patients to demonstrate the safety of etidronate.

If etidronate is effective in increasing the bone mass in AS, it may prevent the dorsal spinal deformity and the eventual spinal fusion which occurs in many patients.

The *aim* of this study was to evaluate the efficacy of etidronate on disease activity, markers of bone turnover and BMD in patients with AS who have mild disease and osteoporosis in a double blind placebo controlled trial.

Methods

Study design

Of the 25 patients selected for the study 7 dropped out prior to randomisation leaving 18 patients (aged 26-52 years), who successfully completed the study. Of these 18 patients, 9 were randomised to the placebo group and 9 to the etidronate group. The duration of the study was 65 weeks. All patients took 500 mgs of calcium daily in the interval when they were not taking the study drug. Patients were instructed to take their medication with water at bedtime, ensuring an interval of at least 2 hours after the last meal. Each patient took the study drug for 2 weeks followed by 11 weeks of calcium. All patients were informed to carry on with their non steroidal anti inflammatory drugs (NSAIDS) as usual. Adverse effects were checked for at every visit. The study was performed as a double blind placebo controlled trial.

Selection of patients

The patients selected for the study fulfilled additional selection criteria besides the ones listed in section 2.2. These are as follows: All patients were required to have early morning stiffness greater than 30 minutes, elevated plasma viscosity or C-reactive protein, and the BMD of either the lumbar spine or femoral neck had to be below 1 SD than the age and sex matched mean.

Clinical assessments

Full clinical and metrological assessment was performed on all patients every 13 weeks. Changes in their clinical status including visual analogue scales for pain in the cervical, thoracic and lumbar spines, early morning stiffness and peripheral joint status were recorded. Metrological assessment included lumbar flexion by the modified Schober's method, chest expansion, tragus to wall distance and intermalleolar distance.

Laboratory investigations

Blood and serum was collected and stored as detailed in section 2.6.

1. The following routine investigations were carried out on each patient at every visit:

- FBC (including white cell count and platelet count),
- Acute phase reactants (plasma viscosity and C-reactive protein),
- Renal function (creatinine, urea and electrolytes),
- Liver function (bilirubin, alkaline phosphatase, γGT, ALT and AST),
- Corrected calcium and phosphate,
- 2 hour urinary calcium/creatinine ratio,

2. The following tests were performed once only at the beginning of the study with the sex hormone profile repeated at the end:

- Thyroid function (TSH),
- Serum protein electrophoresis,
- Sex hormone profile (FSH, LH, Prolactin, Testosterone, SHBG),
- 24 hour urinary calcium and cortisol,

3. All patients had biochemical markers of bone turnover measured as described in section 2.6. These were measured at the start of the study, during the third (32 weeks) and final visits (65 weeks), and included the following:

- Alkaline phosphatase (ALP)
- Bone alkaline phosphatase (BALP)
- Osteocalcin
- Urinary Pyridinoline (Pyr) and Deoxypyridinoline (Dpyr)

Radiological assessments

All patients had radiographic examination of their spines (cervical, thoracic and lumbar), hips and sacroiliac joints at point of entry and at the end of the study. BMD was performed at baseline, on the third visit (32 weeks) and the final visit (65 weeks).

Statistical analysis

Statistical analysis was performed using the Mann Whitney test, Wilcoxon Rank Sum test and the Chi square test. Values were considered to be significant if the p values were < 0.05.

Results

Baseline descriptive characteristics of all patients in the two groups (placebo and etidronate) are shown in Tables 5.1.a, 5.1.b and 5.1.c.

Table 5.1.a shows that the median age of the patients of placebo group was higher than that of the etidronate group at baseline, but the difference was not significant. There was no difference in disease duration or body mass index between the two groups. The disease activity in both groups and the lumbar spine mobility were similar at the commencement of the study.

Table 5. 1. a. *Baseline demographic features, clinical and laboratory indices of disease activity in the placebo and etidronate group.*

Variable	Placebo (n = 9)		Etidronate (n = 9)		
	Median	Range	Median	Range	P value
Age (years)	43	31-52	36	26-44	NS
Duration (years)	10	7-20	11	4.5-18	NS
Body Mass Index	24.8	23.4-29.5	24.8	18.7-32.9	NS
VAS-Cervical spine (cms)	3.5	0-7.5	4.5	0.3-7	NS
VAS-Thoracic spine (cms)	2.5	0-5.7	1.5	0-3.6	NS
VAS-Lumbar spine (cms)	2.5	0.8-6	4	1.5-7.3	NS
Early morning stiffness (min)	75 min	(30min-2hr)	75mins	(30min-2hr)	NS
Tragus-Wall (cms)	10	8.7-15.9	9.75	(9-13)	NS
Chest Expansion (cms)	4.1	2.1-5.9	4.5	3.1-5	NS
Modified Schober's (cms)	7.5	5.5-9.5	7.1	5.5-9.4	NS
Intermalleolar distance(cms)	117	100-129	122	76-145	NS
Plasma Viscosity (m Pas)	1.82	1.72-1.94	1.91	1.68-2.05	NS
C-Reactive Protein (g/l)	0.012	0.01-0.021	0.011	0.01-0.039	NS

Body mass index - [weight (kg)/height (m^2)]

VAS - visual analogue scale.

NS - not significant

The patients of the etidronate group had higher median values of ALP and BALP compared with the placebo group at point of entry into the study, but the difference was not significant (table 5.1.b). No significant difference was observed between any other biochemical or hormonal marker of bone turnover between the two groups at baseline.

Table 5. 1. b. *Baseline descriptive characteristics in biochemical and hormonal markers of bone turnover in the placebo and etidronate groups.*

Variable	Placebo (n = 9)		Etidronate (n = 9)		
	Median	Range	Median	Range	P value
Calcium (mmol/l)	2.27	2.17-2.54	2.28	2.12-2.39	NS
Phosphate (mmol/l)	1.04	0.96-1.17	1.12	0.8-1.35	NS
Testosterone (nmol/l)	14.1	8.1-23	14.3	7.8-20.2	NS
SHBG (nmol/l)	28	25-46	23	(12-70)	NS
25 (OH) Vit D (μmol/l)	20	13-53	22	(10-35)	NS
Parathormone (pmol/l)	3.9	2.1-9.3	2.9	1.8-9.5	NS
Ur. Calcium/creatinine (mmol/l)	0.26	0.14-0.84	0.24	0.03-0.33	NS
24hr urine calcium (mmol/24hr)	5.9	1.3-8.91	4.55	2.1-6.9	NS
Urine free cortisol (nmol/24hr)	130	82-242	133	12.5-252	NS
ALP (U/l)	58	44-98	81	54-101	NS
BALP (U/l)	29	23-49	37	26-53	NS
Osteocalcin (μg/l)	7.06	1.43-9.152	7.35	1.25-9.31	NS
Pyr/Creatinine (nmol/mmol)	9.843	0.73-35.92	7.65	1.3-29.45	NS
Dpyr/Creatinine (nmol/mmol)	3.1	0-8.19	3.49	0.34-9.06	NS

NS - not significant

Table 5.1.c shows no difference in the BMD between the patients of either group at point of entry into the study.

Table 5. 1. c. *Baseline BMD measurements in the placebo and etidronate groups.*

Variable	Placebo (n = 9)		Etidronate (n = 9)		
	Median	Range	Median	Range	P value
Z score					
Lumbar spine	-1.42	-2.96 to (-0.57)	-1.52	-2.98 to (-1.04)	NS
Femoral neck	-1.05	-1.78 to (+0.51)	-1.22	-1.78 to (-0.37)	NS
BMD (gm/cm^2)					
Lumbar spine	0.93	0.77 to 1.1	0.92	0.77 to 0.97	NS
Femoral neck	0.78	0.68 to 0.97	0.78	0.72 to 0.86	NS

NS = not significant.

Effect of Smoking, Alcohol consumption and Exercise in the two groups

Chi square test was used to analyse the difference between the two groups with respect to smoking, alcohol consumption and exercise. No difference was noted in either variable between the two groups.

Change in disease activity

No significant change was observed in any variable in the placebo group before and after treatment (table 5.2). In the etidronate group, significant improvement ($p < 0.05$) was observed in visual analogue scales for pain in the cervical and lumbar spines and in early morning stiffness (table 5.2). No change in the acute phase reactants was seen in either the placebo or the etidronate group.

Table 5. 2. *Change in disease activity in the placebo and etidronate groups following treatment (* = $p < 0.05$).*

Variable	PLACEBO (n = 9)		ETIDRONATE (n = 9)	
	Pre Treatment	*Post Treatment*	*Pre Treatment*	*Post Treatment*
	MEDIAN (Range)	MEDIAN (Range)	MEDIAN (Range)	MEDIAN (Range)
Body Mass Index	24.80 (23.43-29.49)	25.83 (22.44-29.13)	24.76 (18.72-32.91)	26.54 (19.65-29.98)
VAS Cervical spine (cms)	3.50 (0-7.5)	3.50 (0-7.2)	4.50 (0.3-7)	1.00 * (0-5)
VAS Thoracic spine (cms)	2.50 (0-5.7)	2.00 (0-5.6)	1.50 (0-3.6)	1.50 (0.3-2.7)
VAS Lumbar spine (cms)	2.50 (0.8-6)	1.00 (0-7)	4.0 (1.5-7.3)	1.50 * (0-3.2)
Early morning stiffness	2.00 (2-3)	2.00 (0-3)	2.00 (2-5)	1.00 * (0-3)
Tragus-Wall (cms)	10.00 (8.7-15.9)	10.20 (8.5-17.4)	9.75 (9-13)	10.10 (9-12)
Chest Exp (cms)	4.10 (2.1-5.9)	4.00 (1.8-6)	4.50 (3.1-5)	4.80 (3-5)
Lumbar flexion (cms)	7.50 (5.5-9.5)	7.00 (4.2-9.5)	7.10 (5.5-9.4)	7.00 (5-8.5)
Inter Malleolar Distance (cms)	117.00 (100-129)	119.00 (98.5-135)	122.00 (76-145)	115.00 (70.5-144)
Plasma Visc (mPas)	1.82 (1.72-1.94)	1.93 (1.76-1.98)	1.91 (1.68-2.05)	1.82 (1.69-2.19)
C Reactive Protein(g/l)	0.012 (0.01-0.021)	0.011 (0.01-0.04)	0.011 (0.01-0.039)	0.012 (0.01-0.034)

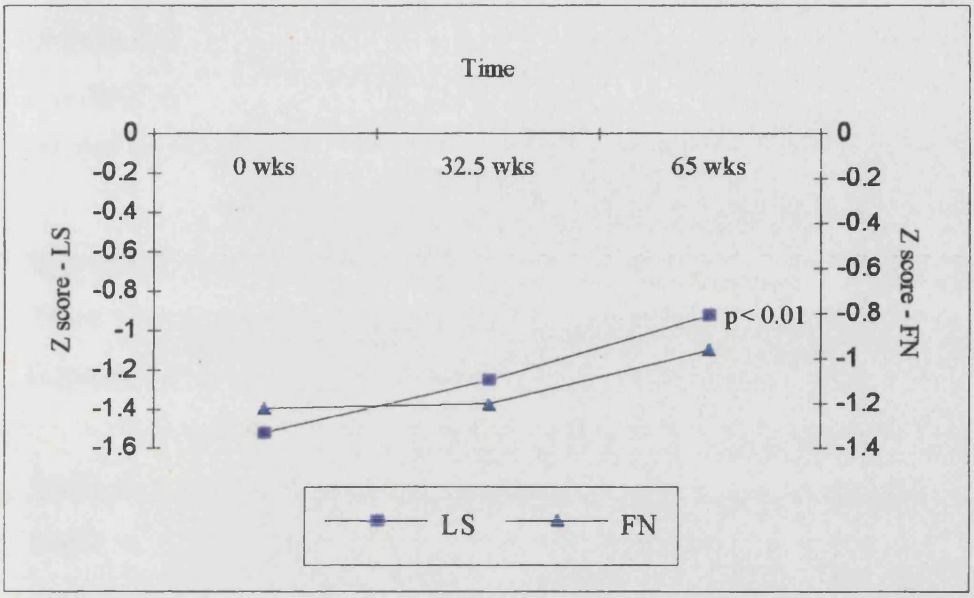
Change in biochemical and hormonal markers

No biochemical or hormonal marker of bone turnover changed significantly in either group following completion of the study.

Change in BMD (median values)

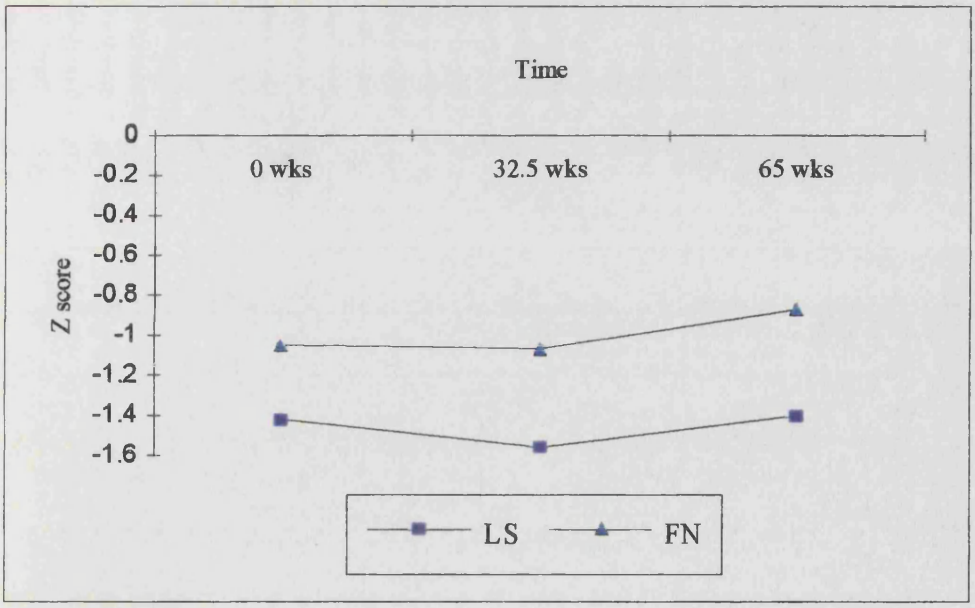
BMD of the lumbar spine in the etidronate group improved significantly ($p<0.01$) following treatment (Fig 5.1). The femoral neck BMD also improved in 7 out of 9 patients, but the improvement was not significant. Patients of the placebo group showed slight but not significant improvement of their lumbar spine and femoral neck BMD (Fig 5.2).

Figure 5. 1. *Sequential changes in median BMD in the Etidronate Group following treatment.*



0 wks - baseline 32.5 wks - third visit 65 wks - final visit

Figure 5. 2. *Sequential changes in median BMD in the Placebo Group following treatment.*



0 wks - baseline 32.5 wks - third visit 65 wks - final visit

Difference between the two groups at the end of study

Disease activity

No significant difference was observed in disease activity between the two groups at the end of the study.

Markers of bone turnover

There was no significant difference in the biochemical and hormonal markers of bone turnover between the two groups at the end of the study.

BMD of the lumbar spine and femoral neck

BMD of the lumbar spine and the femoral neck improved in both groups, but the improvement in BMD at either site was not significant when the two groups were compared at the end of the study (lumbar spine BMD: $p = 0.06$ and femoral neck BMD: $p = 0.08$). The difference in Z scores of the lumbar spine and femoral neck between the two groups over 65 weeks are shown in figures 5.3 and 5.4.

Figure 5. 3. *Difference in median lumbar spine BMD between the two groups*

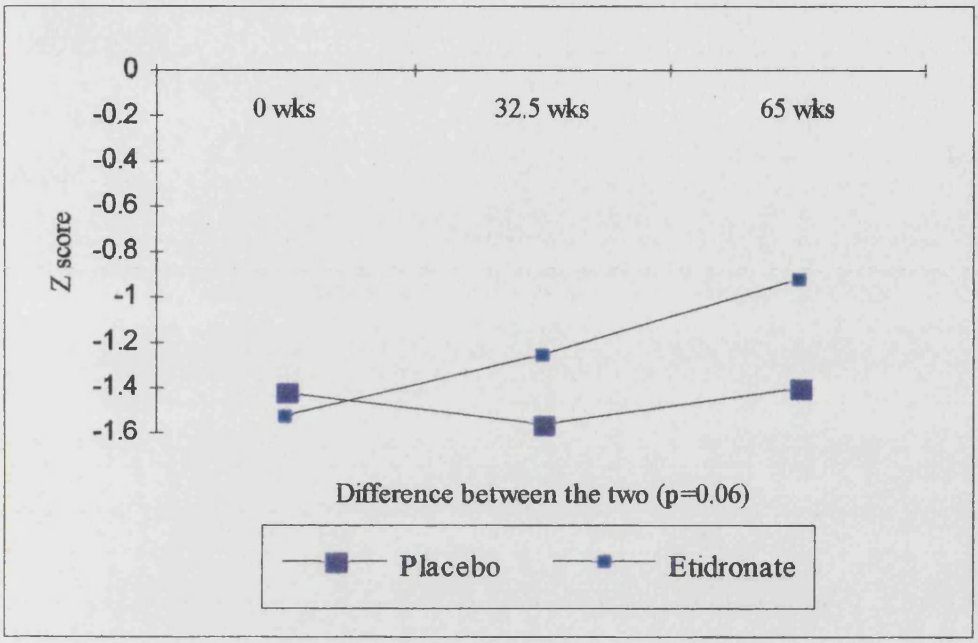
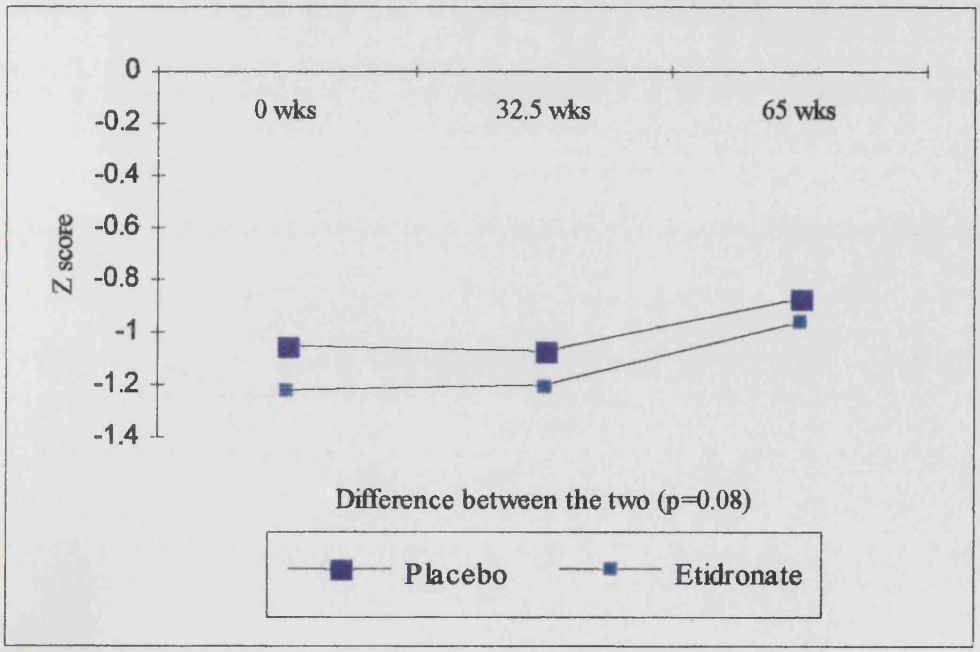


Figure 5. 4. *Difference in median femoral neck BMD between the two groups*



Adverse effects due to etidronate

No adverse effects of etidronate were observed in any patient during the course of the study.

Discussion

Following completion of the study, the patients in the etidronate group showed a significant improvement in their symptoms and BMD of the lumbar spine. They also had minimal increase in their femoral neck BMD, but this change was not significant. Patients receiving placebo also had marginal improvement of their spinal and femoral neck BMD, but no change was observed in their symptoms. Neither group showed any significant change in the biochemical or hormonal markers of bone metabolism.

The fact that the osteoporosis occurs in the femoral neck and spines in the absence of any local pathology at these sites suggests a systemic basis for the osteoporosis. The osteoporosis in patients with AS is mainly trabecular as the cortical areas are spared (Will et al 1989, Devogelaer et al 1992, Mullaji et al 1994). Trabecular bone is metabolically more active and susceptible to hormonal, biochemical and cytokine influences than cortical bone.

The osteoporosis in AS is characterised by low bone formation (Franck et al 1993). This is true of the patients in this study as well (chapter 4). Osteoporosis in AS is characterised by low bone turnover, which is primarily due to a reduction in the number of active bone multicellular units (BMUs) and a reduction in bone formation. Bisphosphonates can cause an improvement in bone mass at the BMUs by decreasing the resorption depth, with a consequent increase in bone mass. This would eventually lead to an increase in bone mass in patients with low turnover osteoporosis. Bisphosphonates may also affect turnover by reducing the production of osteoclast stimulating factor(s) by osteoblasts. (Fleisch 1993).

The significant improvement in BMD of the lumber spine following treatment with etidronate and the minimal effect in the femoral neck is in accordance with results seen with etidronate in studies of postmenopausal osteoporosis (Storm et al 1990, Watts et al 1990). These two studies also demonstrated a decrease in the incidence of vertebral fractures following etidronate therapy. The evaluation of the effect of etidronate on the incidence of vertebral deformities was not possible in this study due to the small number of patients. This was also the reason why a relationship between BMD and

vertebral deformities could not be demonstrated in this cohort of patients. The use of etidronate was however justified in these subjects due to its anti-inflammatory properties and its ability to increase bone mass in patients with low spinal BMD.

The improvement in symptoms in the AS patients is an unexpected and novel finding which needs further investigation. It is possible that the increase in bone mass in the vertebral bodies reverses the microfractures, which might develop as a consequence of osteoporosis, and thus alleviates the symptoms. It is also possible that anti-inflammatory properties of etidronate are responsible for the reversal of symptoms. Studies have reported anti-inflammatory activity of etidronate and clodronate, in adjuvant arthritis models in rats (Flora 1979, Francis et al 1972). In his study Flora (1979) concluded that etidronate and clodronate "inhibited osseous changes, pedal inflammation and the change in body weight gain pattern that is characteristic of adjuvant arthritis models". The anti-inflammatory activity of clodronate was found to be more potent than that of etidronate. Francis et al (1972) in a similar study observed that the anti-inflammatory effect of etidronate was seen in the adjuvant model where there was extensive bone involvement. Neither study however commented on the effect of these medications on acute phase reactants. Moreover, there is no published report on the anti-inflammatory properties of bisphosphonates on arthritides in humans. Therefore it is difficult to be certain about the precise anti-inflammatory effect of bisphosphonates in inflammatory arthritides in humans. In this study as all patients had osteoporosis, it is possible that etidronate did alleviate symptoms of pain and stiffness in the patients with AS. However, no effect was seen on the acute phase reactants. Formal studies of the effect of bisphosphonates on inflammatory arthritides are necessary to evaluate the anti-inflammatory properties of these drugs.

The lack of improvement in the biochemical or hormonal markers is to a certain extent consistent with results of other studies in postmenopausal osteoporosis (Storm et al 1990, Watts et al 1990). The latter two studies however, noted a significant decrease in levels of alkaline phosphatase in the etidronate group. This change was not seen in the present study. The duration of etidronate therapy in this study was 65 weeks, whereas the duration of therapy in the two studies mentioned above was 150 weeks

and 100 weeks respectively. It is possible that changes in the biochemical and hormonal markers of bone turnover may have become more evident had the duration of the study been longer. The small number of patients in this study may also be responsible for the lack of change in biochemical markers.

Although symptoms and BMD of the lumbar spine in the etidronate group improved significantly after 65 weeks of treatment, there was no difference between the two groups at the end of the study. This lack of difference could well be due to the small sample size in the study or due to the low power of the study. An additional factor could be the increase in BMD of the placebo group as a result of the beneficial effects of calcium. It is possible that a greater improvement could have been seen had the duration of the study been longer with a larger population, or had the power of the study been greater. Apprehensions concerning disordered mineralization with etidronate when used for long durations have diminished following the publication of reports on the drug's safety over 5 years (Steiniche et al 1993, Papapoulos et al 1993). It is however worth noting that of all the bisphosphonates, the stongest negative effect on bone mineralization is that of etidronate.

In conclusion, although intermittent cyclical etidronate has a beneficial effect on the BMD of the lumbar spine in patients with AS and osteoporosis, this needs further validation in view of the lack of significant difference between the two groups at the end of the study. Further trials are also necessary to evaluate the beneficial effect of etidronate on the symptoms in patients with AS, particularly in view of the lack of response of the acute phase reactants.

CHAPTER 6 - CONCLUSIONS

The important conclusions that may be drawn from the studies are summarized below. The implications of the results are discussed.

VERTEBRAL DEFORMITY STUDIES

The studies of vertebral deformities in AS emphasized the need to have standardised techniques and methods for their definition. Although several quantitative and semi-quantitative methods have been described, as yet there is no consensus on what constitutes a vertebral fracture. This is mainly due to the absence of a "gold standard" for fracture definition. The reference values on which the algorithms for the definition of deformities are based should be derived from within population groups as considerable variation in vertebral heights exists between poulations (O'Neill et al 1994). It is apparent from the study described in chapter 3 that the prevalence of vertebral deformities can change depending on the method and reference values used.

Need for a gold standard for the definition of vertebral deformities

Although vertebral deformity is a hallmark of osteoporosis, there is no uniformity in the way it should be defined. The two methods used to define vertebral deformities in patients with AS were the methods described by Eastell et al (1991) and McCloskey et al (1993). The criteria these methods employ for the definition of deformities vary, with the McCloskey method using two criteria to define a deformity and the Eastell method only one for a similar deformity. This affects the sensitivity and specificity of the methods and leads to a difference in the prevalence of deformities. It is acknowledged that the prevalence of false positives by the Eastell and McCloskey methods is 12% and 1% respectively when 3 SD is used as a cutoff for fracture definition. Recent guidelines have suggested that prevalent deformities be defined on the basis of a reduction of 3 SD or more from reference values of vertebral height ratios (National Osteoporosis Foundation Working Group on Vertebral Fractures 1993).

The two methods used for the studies described here utilize vertebral height ratios to define fractures and not absolute vertebral heights, thereby minimising errors associated with positioning and projectioning.

Importance of appropriate and adequate reference values for vertebral fracture definition

Vertebral deformities defined by morphometric techniques are based on a comparison between objective measurement of vertebral heights and normal reference values.
The source of these reference values may be from populations that are considered "normal" after the radiographs are assessed qualitatively by a radiologist, or from population samples in which some vertebrae with deformities are included. From the latter, the deformed vertebrae are deleted from the final assessment by trimming them out using the method described by Melton et al (1993).

For the purposes of these studies, the reference values used were derived from 600 UK population based male subjects, recruited during the course of a survey (EVOS), and from within population groups, viz. the AS patients and local controls. All three reference values were derived after trimming the groups (Melton et al 1993) and deleting the deformed vertebrae. This study has shown (chapter 3) that the prevalence of deformities differs quite substantially depending on the reference values being used. The high prevalence of deformity observed when reference values from within population groups are used (AS patients and local controls) is mainly due to the small sample size. When a larger population is used as source for reference values, the prevalence of deformities in AS was similar to that described in other studies (Ralston et al 1990, Donnelly et al 1994). Ideally however, the reference values should be derived from within the population group being studied taking care to ensure that the group is sufficiently large.

Donnelly et al (1994) based their algorithm for fracture definition on normal female vertebral heights due to the non-availability of a male database for this purpose at that time. They felt this was viable because vertebral height ratios were used instead of absolute vertebral heights, thereby decreasing the sex bias. It has, however, been

shown that there is significant variation in vertebral height ratios between sexes (O'Neill et al 1994), hence, the prevalence of deformities derived by using female reference values is likely to be less accurate. The use of a female reference for men is also inappropriate due to the possible differences in the pathogenesis of fractures between men and women.

These studies therefore emphasize the need to use appropriate reference values for the definition of deformities with particular attention being paid to the population group and the number of vertebrae used for the derivation of reference values.

Increased risk of vertebral fractures in AS as compared to healthy controls

The risk for vertebral deformities was increased in patients with AS compared with controls inspite of the latter being of an older age group. Researchers have differed considerably in their views regarding vertebral fractures in AS. The only study to use recommended morphometric techniques to define deformities (Donnelly et al 1994) observed a prevalence of 13% in *male* patients with AS compared with *females* of a similar age range.

In contrast to vertebral deformities, the risk of fractures in the femoral neck and appendicular skeleton in patients with AS has been described to be extremely low even in individuals with advanced disease (Cooper et al 1994). Although the BMD of the appendicular skeleton has been described as normal, that of the femoral neck tends to be low (Will et al 1989, Devogelaer et al 1992, Mullaji et al 1994). In light of these observations, the lack of fractures in the femoral neck needs further evaluation.

Vertebral deformities tend to occur predominantly in the thoracic spine

Ninety five percent of the deformities in the patients with AS described in the studies here occurred in the thoracic spine. These findings agree with observations of Cooper et al (1994) and Donnelly et al (1994). Ralston et al (1990) observed that vertebral deformities were equally distributed throughout the thoracic and lumbar spine. It is

possible that the development of deformities in the thoracic spine at an early stage may in part be responsible for the kyphotic deformity that is characteristic of advanced AS.

Other factors in addition to osteoporosis may be responsible for the vertebral deformities in AS

One problem with the estimates of vertebral fractures derived by morphometric techniques is that not all vertebral deformities reflect a fracture. An analysis of the radiographs of the patients in this study suggests that conditions such as spondylodiscitis and Romanus lesions may also be responsible for the altered shape of vertebral bodies. This could be one reason why no relationship was seen between BMD of the lumbar spine and deformities in AS.

BONE MINERAL DENSITY STUDIES

The established diagnostic criteria for adult women, as determined by the World Health Organisation (WHO), has been set at 2.5 SD below the young adult mean value. Unfortunately no such standard diagnostic criteria have been set for men. For the purposes of this study the cutoff for osteoporosis was considered to be 1 SD below the mean for age and sex matched men. The dual energy x-ray absorptiometer (DXA) used during the course of this study was Hologic QDR 1000.

DXA measurements are useful for the diagnosis of osteoporosis in patients with mild AS.

A large proportion of patients described in these studies had significantly low BMD of the lumbar spine and femoral neck. Since these patients were radiologically characterised by absent or incipient syndesmophytes in their thoracic and lumbar spines and normal hip joints, these results confirmed findings of Will et al (1989), Devogelaer et al (1992) and Mullaji et al (1994). The latter two studies in addition, observed that BMD of the lumbar spine tended to be normal or elevated compared with age matched controls in patients with advanced spinal changes. They concluded that this was due to the presence of syndesmophytes. They also observed low BMD of the femoral neck in mild as well as advanced AS. Donnelly et al (1994) using sequential BMD measurements observed that the BMD of the femoral neck decreased as the disease deteriorated whereas that of the lumbar spine increased, the latter mainly as a result of syndesmophytes in the spine.

The present study (chapter 3), described BMD of the lumbar spine and femoral neck in the largest number of patients with mild disease which suggests that the use of DXA is useful for the diagnosis of osteoporosis in patients with AS who have minimal spinal changes.

BMD of the lumbar spine or femoral neck does not predict the risk of vertebral deformities in patients with AS.

No relationship was seen between BMD of the lumbar spine or femoral neck and vertebral deformities in patients with AS. The BMD was not reduced significantly even in patients with deformities that were more than 4 SD below the normal mean. The results therefore indicate that BMD of the lumbar spine or femoral neck does not predict the risk of vertebral deformities.

BMD of the lumbar spine and proximal hip bears no relationship to markers of bone turnover in AS

No relationship could be demonstrated between markers of bone turnover and BMD of either the lumbar spine or femoral neck in patients with AS. Neither the markers of bone formation nor resorption correlated with BMD.

BONE TURNOVER STUDIES

Markers of bone formation and resorption were evaluated in patients with AS who have mild disease. The decrease in osteocalcin (and bone alkaline phosphatase in a small subgroup) in AS is most likely due to supression of osteoblastic activity by inflammatory mediators. Bone resorption is unaffected in AS.

Osteoporosis associated with AS may be due to low bone formation

Results of the study described in chapter 4 suggests that one of the reasons for the osteoporosis associated with AS is low bone formation which can be attributed to suppression of osteoblastic activity by inflammatory mediators. The disparity seen between osteocalcin and alkaline phosphatase along with low levels of osteocalcin and BALP found only in a small subgroup of men with severe spinal osteopaenia suggests that the decrease in osteoblastic activity is not uniform in all men with AS. The group with the lowest spinal BMD in addition had higher levels of acute phase reactants of inflammation which indicates that certain patients who have active or uncontrolled disease are likely to have greater reduction in bone formation rates. The relationship between inflammatory disease activity and bone formation rates is strengthened by the observation that chronic diseases of non-inflammatory origin have normal levels of osteocalcin (Slovik et al 1984). Besides, levels of the calciotropic hormones [25 (OH) Vit D and PTH] that regulate the synthesis of osteocalcin, were normal (study described in chapter 4) and therefore cannot be the cause of the low osteocalcin.

In addition, a state of relative hypogonadism was observed in the small subgroup of patients with the lowest spinal BMD. This coupled with the correlation between osteocalcin and testosterone suggests that low testosterone levels can further decrease the bone mass in the subgroup of men with AS who have low bone formation.

Bone resorption is unaffected in patients with mild AS

The levels of the urinary collagen crosslinks were not significantly different in patients with AS comapared with controls (described in the study in chapter 4). Neither was the ratio of Pyr to Dpyr significantly elevated. This suggests that bone resorption on the whole is unaffected in mild AS. The low bone mass in AS must therefore occur as a result of low bone formation rates.

Biochemical markers of bone turnover do not predict the risk of vertebral deformity in patients with AS and osteoporosis

No significant relationship was observed between the biochemical markers of bone turnover and vertebral deformities in patients with AS. Although this was true of deformities that were 3 SD below the mean, the level of osteocalcin was significantly reduced in the deformities that were 4 SD below the mean. In light of this finding, it is possible that a significant relationship between deformities and biochemical markers would have been evident had the number of patients in the study been greater.

DRUG STUDIES

The drug administered to patients with mild AS in a double blind placebo controlled study was the first generation bisphosphonate, etidronate. Although the effects of etidronate are known in osteoporosis per se, there is no data on the effects of bisphosphonates on the osteoporosis associated with AS. This study was carried out to evaluate the effects of etidronate on patients with AS who had osteoporosis.

Etidronate can increase lumbar spine BMD in patients with AS who have osteoporosis

The patients of the etidronate group showed significant improvement in the BMD of their lumbar spine at the end of the study in comparison with patients of the placebo group. Although the BMD of the femoral neck improved, the change was not significant. These changes secondary to etidronate therapy were consistent with the changes seen in patients of postmenopausal osteoporosis (Watts et al 1990, Storm et al 1990).

The improvement in the symptoms in patients with AS (chapter 5) may be explained by the anti-inflammatory effects of etidronate observed in adjuvant arthritis models in rats (Flora 1979, Francis et al 1972). It is also possible that by increasing the bone mass etidronate reverses the microfractures or microarchitectural distortion that may be present in patients with AS and thereby alleviates the pain and stiffness associated with this condition. The lack of improvement in the acute phase reactants in response to etidronate needs further evaluation.

Studies evaluating the effects of etidronate in postmenopausal osteoporosis observed a decrease in alkaline phosphatase following therapy (Watts et al 1990, Storm et al 1990). No such change was observed in this study and neither did any other marker of bone turnover alter significantly at the end of the study. This might be due to the small

sample size and a repeat study with a larger number of patients would help to clarify the matter further.

CHAPTER 7 - FURTHER STUDIES

The results of this study leave many unanswered questions regarding the pathophysiology of osteoporosis in AS. Some potential areas for future research are suggested below.

1. Incidence of vertebral deformities in mild AS

It is important to be able to objectively determine the progression of vertebral deformities and establish the incidence of new deformities in sequential radiographs. The studies performed here have shown a greater prevalence of deformities in the thoracic spine (chapter 3) and the beneficial effects of etidronate on bone mass in patients with AS who have mild disease (chapter 5). Two other studies have observed a greater prevalence of deformities in the thoracic spine (Cooper et al 1994, Donnelly et al 1994). It is possible that deformities in the thoracic spine which develop early in the disease process contribute to the kyphosis which is characteristic of advanced AS. If this is so then early identification of deformities and treatment may possibly help to prevent the kyphosis. A longitudinal study using bisphosphonates with sequential radiographs or DXA morphometry of the spine are necessary to establish this.

2. Evaluation of the ability of BMD to predict vertebral fracture risk in AS

In the study described in chapter 3 no relationship between vertebral deformities and BMD was evident. The measurement of BMD in the lumbar spine included not only the vertebral bodies but also the posterior elements of the spine which are involved in the disease process in AS. This could have contributed in part to the lack of relationship seen between BMD and vertebral deformity. Devogelaer et al (1992) using QCT demonstrated a reduction in the trabecular bone density of vertebral bodies in patients with AS. These patients had advanced spinal changes but normal BMD by dual photon absorptiometry. Newer DXA machines are now available that are capable of lateral densitometry and vertebral morphometry of the lumbar spine. Studies evaluating the relationship between lateral (trabecular) BMD of the spine and vertebral

deformity may well improve the predictive ability of BMD for vertebral fractures in ankylosing spondylitis.

3. To evaluate whether cytokines correlate with bone turnover and osteoporosis in AS.

The only studies that have evaluated the effect of cytokines in AS have observed conflicting results. Houssiau et al (1988) observed an increase in the levels of IL-6 in the synovial fluid of patients with AS, but not in their serum, whereas Bilgic et al (1994) demonstrated a significant increase of IL-6 in the serum of AS patients but could not demonstrate a relationship of the cytokine with either disease activity or acute phase reactants.

The expression of cytokines and their relationship with biochemical markers of bone metabolism needs to be established.

4. Histomorphometric evaluation of iliac crest bone biopsy in AS

Histomorphometry with appropriate tetracycline labelling should be performed on iliac crest bone biopsies of two groups of patients with AS: (1) Individuals who do not have osteoporosis and (2) Individuals who have osteoporosis, for comparison of the two groups to evaluate differences.

Histomorphometry will give an indication of bone volume, turnover, mineralization and the structural aspect of bone especially the connections between trabeculae.

5. The effects of bisphosponates in patients with AS and osteoporosis

The double blind placebo controlled study described in chapter 5 demonstrated a significant improvement in the symptoms and BMD of the lumbar spine in the patients who received etidronate therapy. However, no significant change was seen in either the acute phase reactants or markers of bone turnover. In addition, when the placebo and

etidronate groups were compared at the end of the study the differences were not significant.

The patient numbers in the study were small and therefore the study should be repeated with a larger number of patients for at least a further two years to confirm the results. The use of newer bisphosphonates should also be considered.

6. Influence of sex hormones on bone mass and immune response in AS

Biologically available testosterone (testosterone free index) was found to be significantly low in patients with the lowest spinal bone mass. The relationship between sex hormones and the immune response in AS has not been explored and data regarding the level of homones in AS is conflicting, with studies describing low [Tapia-Serrano et al (1991)] high [(Borbas et al (1986) and Dougados et al (1986)] and normal [Spector et al (1988)] levels of testosterone in male patients with AS.

In view of the relative hypogonadism seen in a subgroup of patients, replacement therapy with sex hormones should be considered and would require a placebo controlled study.

7. Similar studies in female patients with AS

Only male patients with AS were recruited for the studies reported. The relationship between sex hormones and bone mass in postmenopausal females is well established. These studies should also be repeated in female patients with ankylosing spondylitis.

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APPENDICES

Appendix 1

ANKYLOSING SPONDYLITIS DATA SHEET

Name.....

Unit No. .....

Address.....

.....

.....

Telephone No. Home..... Work .....

Today's Date ..... Date of birth .....

Duration of AS ..... Height..... Weight.....

Past medical history of note .....

.....

Family history .....

Current drug treatment .....

.....

Previous drug treatment (in particular calcium, vitamin D, fluoride, androgens, estrogens, corticosteroids, bisphosphonates, thyroxine) .....

.....

Previous in patient physiotherapy courses yes / no ..... number .....

EXERCISES

Do you do any formal exercises for your AS?

If YES

How many hours/minutes per day do you exercise ?.....

If NO

Do you do any other form of exercise ? Please mention what and for how long / how frequently do you do this ? .....

SMOKING

Do you smoke ?

If YES .....

Cigarattes ..... Cigars ..... Tobacco .....

How many cigarattes / cigars or how much tobacco do you smoke per day ?

.....

Since how long have you been smoking? .....

If NO .....

Are you a NON smoker ? .....

Are you an EX smoker ? If so,

When did you stop ? .....

When had you started smoking ? .....

How much did you smoke at that time ? .....

ALCOHOL

Do you consume alcohol ? YES ..... NO .....

If YES

Approximately how many units of alcohol do you drink in a week ? .....

If NO

Did you drink before ? YES .....NO.....

If YES

When did you give up drinking ? .....

How long ago had you started drinking ? .....

How many units per day did you drink at that time ? .....

1. Physical Assessment

1. Rate the degree of pain you have experienced in your neck over the last week on this scale.

no pain .....unbearable pain

2. Rate the degree of pain you have experienced in your thoracic spine (indicate on the patient) or chest wall, over the last week.

no pain .....unbearable pain

3. Rate the degree of pain you have experienced in your lunbar spine (indicate on the patient) or buttocks, over the last week.

no pain .....unbearable pain

4. List joints which have been painful in the last week .....

.....

5. Rate the degree of pain in these joints in the last week.

no pain .....unbearable pain

no pain .....unbearable pain

no pain .....unbearable pain

no pain .....unbearable pain

6. Rate the average duration of early morning stiffness you have experienced in your spine from the time you first wake up, over the last week.

0	no morning stiffness	3	1 -2 hrs
1	< 30 minutes	4	2 - 4hrs
2	30 minutes - 1 hr	5	> 4 hrs

7. Can you touch your toes with your knees straight ?

0	no difficulty	4	unable to do
1	mild difficulty	4(i)	How long have you been unable to do this (yrs) ? .....
2	moderate difficulty	5	never able to
3	severe difficulty		

8. Do you have any difficulty moving your head and neck (because of pain or restricted movement) ?

0	no difficulty	3	severe difficulty
1	mild difficulty	4	unable to do
2	moderate difficulty		

9. List the painful joints which are inflammed (increased warmth and swelling)

.....

.....

10. List and document the joints where the ROM is restricted.

.....

.....

11. Modified Schober's (cm) .....

12. Metrology (list in card)

CONSENT FORM

I hereby give my consent to participate in this study. I have read and understood the patient information sheet and my doubts have been cleared by Dr D Mitra.

Name .....

Address

.....

.....

.....

Signature .....

Date .....

Signature of witness .....

Appendix 2

Reference values for vertebral deformities

Appendix 2. a.

UK reference values

Height Ratio	Untrimmed		Trimmed		Threshold	
	Mean	SD	Mean	SD	Grade 1	Grade 2
Anterior						
T 4	94.11	5.72	94.12	5.04	79.01	73.98
T 5	91.65	6.03	91.85	5.57	75.13	69.55
T6	89.97	6.1	90.07	5.31	74.12	68.81
T 7	89.43	6.61	89.5	5.76	72.22	66.46
T 8	90.79	7.92	90.85	7.28	69.02	61.74
T 9	93.96	8.84	93.93	7.7	70.83	63.13
T 10	96.73	8.66	96.95	8.11	72.62	64.51
T 11	94.25	8.46	94.22	8.22	69.56	61.34
T 12	91.69	8.64	91.37	6.96	70.51	63.55
L 1	91.34	7.38	91.48	6.46	70.1	65.64
L 2	96.09	6.31	96.06	5.34	80.04	74.7
L 3	100.08	6.54	99.93	5.96	82.04	76.08
L 4	104.19	7.58	104	6.92	83.24	76.32
Central						
T 4	91.49	4.68	91.6	4.15	79.15	75
T 5	91.22	4.76	91.35	4.03	79.27	75.24
T 6	90.95	4.67	90.98	4.18	78.45	74.28
T 7	91.3	4.5	91.35	3.96	79.46	75.5
T 8	91.38	4.93	91.4	4.28	78.57	74.3
T 9	92.28	4.91	92.3	4.55	78.64	74.08
T 10	92.61	4.95	92.7	4.55	79.04	74.48
T 11	91.52	5.08	91.59	4.73	77.41	72.69
T 12	90.81	5.12	90.83	4.48	77.39	72.91
L 1	89.84	5.34	90.05	4.15	77.6	73.44
L 2	89.64	5.37	89.74	4.39	76.57	72.18
L 3	91.61	5.13	91.58	4.82	77.11	72.28
L 4	96.46	5.49	96.53	5.1	81.24	76.14

Table continued overleaf

Height Ratio	Untrimmed		Trimmed		Threshold	
	Mean	SD	Mean	SD	Grade 1	Grade 2
Posterior up						
T 5	103.66	5.17	103.6	4.8	89.18	84.38
T 6	102.57	5.19	102.49	4.4	89.27	84.87
T 7	101.3	5.01	101.16	4.14	88.75	84.61
T 8	100.91	5.13	100.88	4.66	86.91	82.25
T 9	101.67	5.04	101.56	4.63	87.67	83.03
T 10	104.69	5.45	104.72	5.41	88.48	83.07
T 11	107.88	6.12	107.37	5.15	91.9	86.75
T 12	108.85	6.7	108.11	5.77	90.8	85.03
L 1	106.3	6.39	105.96	5.75	88.71	82.96
L 2	100.86	4.23	100.83	3.6	90.03	86.43
L 3	99.36	4.26	99.29	3.74	88.08	84.35
L 4	96.06	4.57	95.99	4.29	83.12	78.83
Posterior down						
T 4	96.71	4.86	96.71	4.46	83.32	78.86
T 5	97.74	4.87	97.71	4.2	85.1	80.89
T 6	98.96	4.89	98.91	4.07	86.69	82.62
T 7	99.36	5.13	99.19	4.54	85.57	81.03
T 8	98.61	5.95	98.51	4.56	84.82	80.26
T 9	95.78	5	95.67	4.86	81.1	76.24
T 10	92.99	5.14	93.24	4.59	79.48	74.89
T 11	92.21	5.53	92.37	5.11	77.03	71.91
T 12	94.41	5.58	94.43	5.21	78.78	73.57
L 1	99.32	4.22	99.24	3.51	88.7	85.19
L 2	100.83	4.3	100.76	3.81	89.34	85.53
L 3	104.34	4.94	104.25	4.6	90.44	85.83

Untrimmed - inclusive of all vertebrae

Trimmed - fractured vertebrae deleted

Threshold - threshold values for vertebral deformity
Grade 1 threshold = trimmed mean minus 3 SD, and,
Grade 2 threshold = trimmed mean minus 4 SD.

Appendix 2. b.

AS reference values - decision thresholds

Height Ratio	Untrimmed		Trimmed		Threshold	
	Mean	SD	Mean	SD	Grade 1	Grade 2
Anterior						
T 4	94.28	4.64	94.85	3.85	83.3	79.45
T 5	93.61	5.3	94.6	3.47	84.2	80.73
T6	93.85	5.59	95.08	3.46	84.71	81.25
T 7	91.87	6.24	93.26	3.97	81.33	77.36
T 8	92.48	6.97	94.12	4.77	79.8	75.03
T 9	91.51	7.03	93.31	4.71	79.18	74.47
T 10	93.22	5.11	93.44	4.82	79	74.18
T 11	93.56	4.9	93.56	4.9	78.85	73.95
T 12	92.41	6.59	92.68	6.26	73.88	67.61
L 1	92.7	5.84	93.17	5.27	77.35	72.08
L 2	96.18	5.58	96.18	5.58	79.46	73.88
L 3	98.82	4.56	98.09	2.86	89.51	86.65
L 4	101.67	5.04	101.67	5.04	86.54	81.49
Central						
T 4	92.26	5.43	92.26	5.44	75.95	70.51
T 5	92.28	5.56	92.97	4.63	79.09	74.46
T 6	92.94	4.75	92.92	4.3	80.02	75.72
T 7	91.87	4.86	92.48	4.03	80.39	76.35
T 8	91.53	5.57	91.52	5.13	76.13	71
T 9	91.06	6.58	91.06	6.58	71.31	64.72
T 10	91.32	6.4	91.99	4.71	77.83	73.11
T 11	90.85	6.54	91.78	4.96	76.91	71.95
T 12	91.02	4.95	91.02	4.95	76.17	71.22
L 1	90.77	5.71	90.77	5.71	73.64	67.94
L 2	89.2	4.56	89.41	4.27	76.6	72.33
L 3	90.72	4.1	90.72	4.1	78.42	74.32
L 4	94.73	7.36	95.49	4.08	83.26	79.18

Table continued overleaf

Height Ratio	Untrimmed		Trimmed		Threshold	
	Mean	SD	Mean	SD	Grade 1	Grade 2
Posterior up						
T 5	103.41	4.9	102.29	3.34	92.28	88.94
T 6	102.32	4.76	101.71	3.34	91.68	88.34
T 7	102.57	4.1	102.57	4.1	90.27	86.18
T 8	101.47	4.39	101.9	3.18	92.38	89.2
T 9	104.7	6.3	103.58	4.57	89.87	85.3
T 10	103.97	5.11	103.97	5.11	88.63	83.52
T 11	107.09	5.48	106.59	4.79	92.22	87.43
T 12	109.51	7.39	109.79	7.1	88.5	81.4
L 1	105.95	8.04	105.39	5.09	90.13	85.05
L 2	102.03	4.54	100.8	2.44	93.49	91.06
L 3	99.12	4	99.3	3.76	88.02	84.26
L 4	96.84	4.2	96.83	4.2	84.23	80.03
Posterior down						
T 4	96.91	4.39	97.86	3.2	88.26	85.06
T 5	97.94	4.44	98.42	3.22	88.76	85.53
T 6	97.65	3.96	97.65	3.96	85.77	81.82
T 7	98.74	4.37	98.22	3.04	89.11	86.07
T 8	95.83	5.46	96.73	4.25	83.97	79.72
T 9	96.41	4.72	96.2	4.43	82.9	78.47
T 10	93.61	4.68	94	4.21	81.37	77.16
T 11	91.72	6.15	91.45	5.8	74.05	68.25
T 12	94.88	6.81	94.54	5.19	78.97	73.78
L 1	98.2	4.21	99.26	2.39	92.11	89.72
L 2	101.05	4.13	100.85	3.81	89.41	85.6
L 3	103.46	4.42	103.46	4.42	90.2	85.78

Untrimmed - inclusive of all vertebrae

Trimmed - fractured vertebrae deleted

Threshold - threshold values for vertebral deformity

Grade 1 threshold = trimmed mean minus 3 SD, and,

Grade 2 threshold = trimmed mean minus 4 SD.

Appendix 2. c.

Control reference values

Height Ratio	Untrimmed		Trimmed		Threshold	
	Mean	SD	Mean	SD	Grade 1	Grade 2
Anterior						
T 4	92.06	4.87	92.06	4.87	77.45	72.58
T 5	90.98	4.31	91.27	3.96	79.38	75.42
T6	88.86	4.77	89.23	4.24	76.5	72.26
T 7	87.78	4.73	87.78	4.73	73.58	68.84
T 8	88.16	6.11	88.51	4.66	74.54	69.88
T 9	89.93	5.29	89.93	5.29	74.08	68.79
T 10	90.88	5.77	91.19	4.4	77.99	73.59
T 11	87.37	5.55	87.38	4.83	72.9	68.08
T 12	87.15	5.64	87.15	5.64	70.22	64.58
L 1	88.61	4.67	88.99	4.09	76.71	72.62
L 2	92.65	4.27	92.65	4.27	79.84	75.57
L 3	95.07	4.62	95.07	4.62	81.21	76.59
L 4	96.67	5.9	96.67	5.9	78.97	73.06
Central						
T 4	90.9	4.74	90.9	4.74	76.69	71.96
T 5	90.06	4.15	90.06	4.15	77.62	73.48
T 6	90.62	3.74	90.62	3.74	79.42	75.68
T 7	90.33	3.48	90.33	3.48	79.9	76.42
T 8	90.38	3.89	90.74	3.21	81.11	77.9
T 9	90.88	4.34	90.88	4.34	77.85	73.51
T 10	90.3	4.9	90.3	4.9	75.62	70.72
T 11	88.38	3.6	88.38	3.6	77.6	74.01
T 12	89.6	3.78	89.9	3.31	79.97	76.66
L 1	90.26	3.11	90.04	2.84	81.5	78.66
L 2	90.46	3.14	90.46	3.14	81.04	77.9
L 3	92.29	3.62	92.3	2.76	84.01	81.25
L 4	97.48	4.3	97.48	4.31	84.56	80.25

Table continued overleaf

Height Ratio	Untrimmed		Trimmed		Threshold	
	Mean	SD	Mean	SD	Grade 1	Grade 2
Posterior up						
T 5	104.33	5.27	104.33	5.27	88.52	83.25
T 6	102.44	3.84	102.13	3.39	91.97	88.58
T 7	101.17	3.9	101.09	2.41	93.87	91.46
T 8	102.15	3.97	101.83	3.14	92.41	89.27
T 9	103	4.14	103	4.14	90.59	86.45
T 10	106.02	5.74	105.99	4.94	91.17	86.23
T 11	108.14	4.4	107.77	3.81	96.33	92.51
T 12	104.95	4.35	104.31	3.46	93.94	90.49
L 1	103.87	4.84	104.44	2.76	96.16	93.4
L 2	100.69	2.98	100.47	2.68	92.42	89.75
L 3	99.32	2.5	99.37	2.18	92.83	90.65
L 4	97.67	4	97.67	4	85.67	81.67
Posterior down						
T 4	96.1	4.97	96.1	4.97	81.18	76.21
T 5	97.76	3.64	98.02	3.29	88.15	84.87
T 6	98.99	3.81	98.98	2.37	91.87	89.5
T 7	98.04	3.73	98.3	2.99	89.32	86.32
T 8	97.24	3.94	96.93	3.47	86.51	83.04
T 9	94.58	5.12	94.23	4.02	82.18	78.16
T 10	92.62	3.68	92.9	3.28	83.05	79.77
T 11	95.44	3.87	95.97	3.21	86.34	83.13
T 12	96.48	4.62	95.81	2.51	88.29	85.78
L 1	99.4	2.91	99.6	2.66	91.63	88.97
L 2	100.74	2.58	100.68	2.23	93.99	91.76
L 3	102.55	4.21	102.55	4.21	89.93	85.73

Untrimmed - inclusive of all vertebrae

Trimmed - fractured vertebrae deleted

Threshold - threshold values for vertebral deformity

Grade 1 threshold = trimmed mean minus 3 SD, and,
Grade 2 threshold = trimmed mean minus 4 SD.

ABSTRACTS AND PRESENTATIONS ARISING FROM THIS WORK

1. Osteoporosis associated with ankylosing spondylitis. Mitra D, Ring EFJ, Bhalla AK, Collins AJ (1994). In Ring EFJ (ed): Current Research in Osteoporosis and Bone Mineral Measurement III: 42-43. London, British Institute of Radiology.

2. Biochemical markers of bone turnover in early AS. Mitra D., Dixey J, Dunphy J, Elvins D, Collins AJ, Bhalla AK. Submitted for the American College of Rheumatology conference, 1995.

3. Osteoporosis in early AS: Quantitative measurement by DXA. Mitra D, Elvins D, O'Neill T, Collins AJ, Bhalla AK. Submitted for the American College of Rheumatology conference, 1995.

4. The efficacy of etidronate on disease activity, markers of bone turnover, and BMD in patients with early AS and osteoporosis. Mitra D, O'Neill T, Elvins D, Collins AJ, Bhalla AK. Submitted for the American College of Rheumatology conference, 1995.

5. Prevalence and distribution of vertebral fractures in early AS. Mitra D, O'Neill T, Varlow J, Elvins D, Collins AJ, Bhalla AK. Submitted for the American College of Rheumatology conference, 1995.